Pediat. Res. *1:* 39–49 (1967)

Coagulation kidney
endotoxin rabbits
Shwartzman reaction

Quantitative Aspects of Blood Coagulation in the Generalized Shwartzman Reaction

1. Effects of Variation of Preparative and Provocative Doses of *E. Coli* Endotoxin

J. J. CORRIGAN, Jr.[1], C. F. ABILDGAARD, JANE F. VANDERHEIDEN and I. SCHULMAN

Department of Pediatrics, University of Illinois College of Medicine,
Chicago, Ill. 60612, USA

Extract

The generalized Shwartzman reaction (GSR) is induced in rabbits by two properly spaced intravenous injections of bacterial endotoxin and is characterized by the occurrence of bilateral renal cortical necrosis. This report describes the quantitative changes in the coagulation mechanism in response to variation of preparative and provocative doses of *E. coli* endotoxin and of the interval between injections. It also correlates the coagulation changes with the pathologic findings of renal cortical necrosis.

Renal cortical necrosis was induced in 34 of 49 (70 %) of rabbits using 0.100 mg/kg of endotoxin as the preparative (first) injection and 0.200 mg/kg 24 hours later as the provocative (second) dose. Four hours after the preparative injection white blood cells and platelets fell significantly while fibrinogen, prothrombin time, and Factors V and VIII fell slightly. At 24 hours all determinants except the platelets had recovered and the white cells, Factor V and fibrinogen had risen to significantly higher levels than baseline. Following the second (provocative) injection of endotoxin, a precipitous fall in all factors occurred. Maximal changes developed two hours after the second injection for white cells and platelets and by four hours for the coagulation factors; at this time kidneys show fibrin. By 48 hours all factors except the platelets had returned to 24 hour levels (table I). Rabbits given 0.100 mg/kg of endotoxin and normal saline 24 hours later did not develop cortical necrosis of kidneys or reduction in levels of any of the coagulation factors or platelets (table II).

With 0.1 mg/kg of endotoxin as the preparative dose, the effect of variation in the provocative dose at 24 hours was studied. With 0.2, 0.1, 0.05 mg/kg provocation renal cortical necrosis was observed in 60–100 % of animals. Rabbits given 0.01 mg/kg of endotoxin, or saline, as the second injection did not develop the GSR. Coagulation changes were measured four hours after the provocative injections. With doses of endotoxin of 0.025 mg/kg and greater there was a significant fall in white cells, platelets and each of the coagulation factors measured. With 0.01 mg/kg there was a significant decrease in Factors II, V and VIII but no significant fall in white cells, platelets or fibrinogen. With saline all of the measured factors rose (table V). Changes in fibrinogen following different provocative doses are demonstrated in fig. 5. Renal cortical necrosis did not occur until a fall in fibrinogen of 66 mg/100 ml was produced.

The effect of varying the preparative dose was studied. Groups of rabbits were given either saline, 0.001 mg/kg, 0.01 mg/kg or 0.1 mg/kg endotoxin preparation and all animals received 0.1 mg/kg endotoxin 24 hours later. With saline or 0.001 mg/kg endotoxin as preparation no GSR occurred.

[1] Present address: Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30303, USA.

With a 10 and 100 fold increase in concentration of endotoxin the GSR was regularly produced. With saline preparation significant changes occurred only in white cells and platelets. A significant decline in all factors except Factor VIII was produced by preparation with 0.001 mg/kg endotoxin; the fibrinogen fell 89 mg/100 ml but no animal developed renal cortical necrosis. The inadequately prepared animal appeared able to tolerate an amount of fibrinogen conversion that would, in the properly prepared animal, result in renal cortical necrosis. In rabbits prepared with 0.01 mg/kg and 0.1 mg/kg of endotoxin significant changes occurred in all factors and renal cortical necrosis was produced (table VI). Rabbits were given 0.1 mg/kg endotoxin as preparation and a dose of 0.1 mg/kg was administered at 24 hours to one group of animals and at 48 hours in a second group. In the 24 hour group the GSR occurred in 10 of 13 animals; following endotoxin at 48 hours renal cortical necrosis was not produced in any of 18 rabbits. Changes in the coagulation mechanism four hours after provocation are shown in table VII and demonstrate that the fall was similar in both groups for all factors except fibrinogen. Fibrinogen decreased 178 mg/100 ml in animals given injections 24 hours apart but there was only a 70 mg/100 ml fall in the 48 hour group. The data suggest that by 48 hours after adequate preparation the RES has recovered to the degree that clotting intermediates are removed and significant amounts of fibrin are cleared thus preventing deposition in the kidneys and cortical necrosis.

The interdependence of preparative and provocative doses of endotoxin was demonstrated. A dose of 0.01 mg/kg prepared the animal for subsequent provocation with 0.1 mg/kg; 0.1 mg/kg prepared animals for subsequent provocation with as little as 0.025 mg/kg. However, the combination of 0.01 mg/kg as preparation and 0.025 mg/kg as provocation did not result in cortical necrosis of the kidneys or in a fall of any of the coagulation factors (tables VIII and IX).

Conclusions: In the presence of adequate preparation provocative doses of endotoxin capable of inducing cortical necrosis of the kidneys were always associated with significant consumption of all coagulation factors measured. With inadequate preparation, produced either by decreasing the preparative dose of endotoxin or by increasing the interval between injections, significant activation of the clotting system occurred but renal cortical necrosis did not develop.

Speculation

The development of the generalized Shwartzman reaction, and, by inference, Shwartzman-like human syndromes, appears to require activation of the coagulation mechanism and the development of intravascular clotting. However, the degree of clotting induced by stimuli of known potency and the degree of fibrin deposition in the tissues are critically dependent upon the prior state of preparation of the animals, probably reflecting the functional adequacy of the reticulo-endothelial system.

In recent years there has been increasing recognition of the role of intravascular clotting in the pathogenesis of a variety of disorders [1, 2, 3, 4, 5], many of which have been compared to the generalized Shwartzman reaction (GSR) [6]. The latter is induced in rabbits by two properly spaced intravenous injections of bacterial endotoxin and is characterized by the occurrence of bilateral renal cortical necrosis. Previous investigations have shown that each injection of endotoxin activates the clotting mechanism. However, the first apparently produces only transient injury to the reticuloendothelial system (RES) so that clearance of circulating endotoxin [7], clotting intermediates [8], and fibrin [9,10] is efficiently performed. The second injection of endotoxin, on the other hand, leads to massive intravascular formation of fibrin which cannot be removed due to a now severely impaired RES [11] and, in the rabbit, an ineffective fibrinolytic system [12, 13].

It has been shown by others that the concentration of endotoxin used for each injection and the interval between doses are critical in producing the GSR [14, 15, 16]. However, quantitative coagulation measurements have not been made under these experimental conditions.

This report will describe the quantitative changes in the coagulation mechanism in response to variation of preparative and provocative doses of *E. coli* endotoxin and of the interval between injections. The correlation of the coagulation changes with the pathologic finding of bilateral renal cortical necrosis will also be indicated.

Materials and Methods

1. Rabbits, 1.0 kilogram white hybrid of either sex were used. The animals were housed in an air-conditioned animal room, fed Purina pellets and water, *ad. lib.* Rabbits used in these experiments were free of obvious illness.

2. *E. coli* endotoxin 0127:B8 Boivin type (Difco, Detroit, Michigan). Endotoxin solutions were prepared fresh daily in pyrogen free 0.9 % NaCl (Saline). The appropriate amount was injected via marginal ear vein in a constant volume of 1.5 cc.

3. All studies were performed on blood obtained by cardiac puncture utilizing a two syringe (plastic) technique and short beveled No.18 disposable needles. The blood obtained in the first syringe was used immediately for white blood cell and platelet counts. The second syringe contained 3.8 % sodium citrate as the anticoagulant in the proportion of one volume of anticoagulant to nine volumes of whole blood.

4. Plasma was obtained by centrifugation in plastic tubes for 20 minutes at 5000 rpm in a refrigerated centrifuge. All determinations were performed on the day of cardiac puncture except fibrinogen and Factor VIII. Aliquots of plasma for the latter were frozen immediately and stored for not longer than 48 hours at —20° C.

5. White blood cell counts were done using standard hematologic techniques modified by utilizing Unopette (Becton, Dickinson and Co. Rutherford, N.J.) as the collecting and diluting system. Platelet counts were done by phase microscopy [17]. Prothrombin time (PT) was determined by the method of QUICK [18], prothrombin assay (Factor II) by the method of OWREN and AAS [19], fibrinogen by the method of RATNOFF and MENZIE [20], Factor VIII (AHF) assay employing the partial thromboplastin time by the method of ABILDGAARD *et al.* [21], and Factor V by the method of BIGGS and MCFARLANE [22]. For the latter test Factor V deficient substrate plasma was prepared by absorbing normal human oxalated plasma with 50 mg/ml of Filter-Cel (Johns Manville, Joliet, Illinois) for two hours. The plasma-Filter-Cel mixture was then centrifuged at 10,000 rpm for 10 minutes and the plasma placed in a glass test tube, allowed to age in a 37° C water bath until the one stage prothrombin time was over 25 to 30 seconds.

The white blood cell and platelet counts are expressed as cells/mm^3, the prothrombin time, Factors II, V, and VIII as percent activity as compared to a normal human standard, and fibrinogen as mg/100 ml plasma.

6. In preliminary experiments it was noted that cardiac puncture was associated with a high mortality. This necessitated the use of two groups of rabbits in each experiment. That is, one group was given the two spaced injections of endotoxin but was not subjected to blood sampling in order to permit development of renal cortical necrosis and the determination of incidence. In the second group, which received identical injections of endotoxin, blood was obtained for coagulation studies by cardiac puncture at the intervals indicated in the individual experiments.

7. All animals that died were autopsied. Surviving rabbits were sacrificed 24 hours after the provocative (second) injection of endotoxin. Rabbits were considered to have the GSR when the kidneys demonstrated gross cortical necrosis with or without massive hemorrhage.

8. The results are tabulated as mean values, standard error of the mean and the number of animals. The test for significance, where indicated, was performed by calculating the 't' value for non-paired experiments. A 'p' value of less than 0.05 was considered significant.

Results

When blood clots in a test tube platelets, fibrinogen, prothrombin and Factors V and VIII are consumed. Extensive clotting *in vivo* is likewise manifested by thrombocytopenia and reduced levels of fibrinogen, prothrombin and Factors V and VIII. Therefore, these factors were measured in a series of experiments designed to study, a) the pattern of coagulation changes during the evolution of the typical GSR; b) the effects of variation in the preparative and the provocative doses of endotoxin; and c) the influence of variation in time interval between injections. Changes in the white blood cells were also studied.

A. Coagulation Changes Over a 48-Hour Period During the Evolution of the Typical GSR

In preliminary experiments bilateral renal cortical necrosis was induced in 34 of 49 (70 %) rabbits using 0.100 mg/kg of endotoxin as the preparative (first) injection and 0.200 mg/kg 24 hours later as the provocative (second) dose. It was thus established that these concentrations of endotoxin were adequate for both preparation, and for provocation of the GSR.

In separate groups of rabbits given the same amounts of endotoxin as in the preceding paragraph blood was collected by cardiac puncture at the following times; 0 (before endotoxin), 4, 24, 26, 28, and 48 hours after the first injection of endotoxin. (The second dose of endotoxin was, therefore, administered immediately following the 24-hour cardiac puncture.) The results are shown in table I and figs. 1 and 2. Four hours after the preparative injection the white blood cells and platelets fell significantly, the prothrombin time, and Factors V and VIII fell slightly, while fibrinogen re-

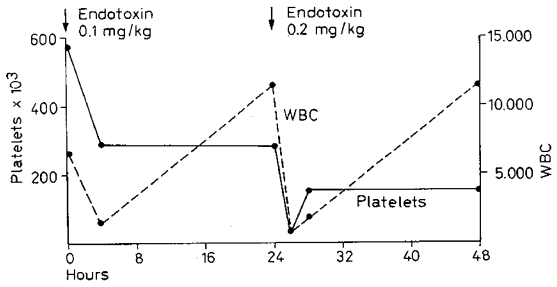

Fig. 1. Mean values for the white blood cells and platelets in rabbits receiving 0.100 mg/kg endotoxin for preparation and 0.200 mg/kg endotoxin at 24 hours for provocation.

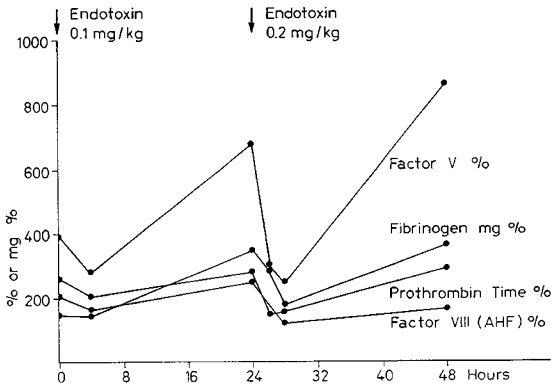

Fig. 2. Mean values for Factor V, Factor VIII, the prothrombin time and fibrinogen in animals prepared with 0.100 mg/kg endotoxin and 0.200 mg/kg endotoxin for provocation at 24 hours. A significant fall in all factors occurred between 24 and 28 hours (or 4 hours following the provoking dose of endotoxin).

mained constant. At 24 hours all determinants except the platelets had recovered and indeed the white blood cells, Factor V and fibrinogen had risen to significantly higher levels than baseline. Following the second or provocative injection of endotoxin a precipitous and highly significant fall in all factors occurred. Maximal changes developed two hours after the second injection for the white blood cells and platelets and by 4 hours for the coagulation factors. At this time kidneys of animals developing cortical necrosis will begin to show fibrin [13, 15]. By 48 hours all factors, except the platelets, had returned to the 24-hour levels.

Twenty rabbits given 0.100 mg/kg of endotoxin preparation and normal pyrogen free saline solution 24 hours later did not develop cortical necrosis of the kidneys. Coagulation studies performed in a similar group of animals are shown on table II and figs. 3 and 4. It will be seen that injection of saline twenty-four hours after endotoxin preparation is not followed by reduction in levels of any of the coagulation factors or of the platelets.

Table I. Coagulation changes in animals receiving 0.1 mg preparation and 0.2 mg provocation of *E. coli* endotoxin

Time (hours)	0	4	24	26	28	48
WBC/mm³						
mean	6300	1500	12500	0820	1650	11400
S.E.	±600	±80	±1376	±31	±48	±1700
(No. animals)	(9)	(5)	(11)	(6)	(6)	(11)
Platelets	590	285	259	48	164	150
$\times 10^3$/mm³	±45	±59	±45	±12	±23	±10
	(13)	(5)	(16)	(6)	(10)	(11)
Factor VIII	215	160	250	159	124	164
%	±20	±23	±14	±34	±19	±19
	(14)	(5)	(14)	(7)	(10)	(11)
Factor V	382	269	680	305	250	874
%	±20	±53	±66	±63	±32	±57
	(14)	(5)	(12)	(6)	(9)	(11)
Prothrombin	268	202	284	151	168	287
time	±16	±35	±10	±12	±14	±10
%	(14)	(5)	(15)	(7)	(10)	(10)
Fibrinogen	170	168	351	287	179	365
mg/100 ml	±6	±16	±22	±32	±21	±24
	(14)	(5)	(15)	(6)	(10)	(10)

Since the experiments described above demonstrated that significant coagulation changes occurred four hours following the provocative injection of endotoxin, attention was directed to the changes in this interval in the experiments to follow. To permit comparison of the data of individual experiments results are expressed as percent change between values found at 24 hours after the preparative injection and those four hours after the provocative injection (28 hours after preparation). The data of the two preceding experiments are expressed in this manner in table III. Thus, in animals prepared with 0.100 mg/kg of endotoxin, provocation with saline was followed by an increase in the measured factors four hours after injection; provocation with 0.200 mg/kg of endotoxin, in contrast, induced a 86 % fall in the white blood cells, 39 % in platelets, 50 % in Factor VIII, 60 % in Factor V, 44 % in the prothrombin time and 49 % (172 mg/100 ml) in fibrinogen. The data indicate, therefore, that in the evolution of the endotoxin-induced GSR the coagulation mechanism is activated in its entirety and that consumption of 172 mg/100 ml of fibrinogen in this system will result in the development of cortical necrosis of the kidneys.

Table II. Coagulation changes in animals receiving 0.1 mg endotoxin preparation and saline provocation

Time(hours)	0	4	24	28	48
WBC/mm^3					
mean	6300	1500	11200	11400	16000
S.E.	±600	±80	±1200	±2700	±1400
(No. animals)	(5)	(5)	(6)	(5)	(6)
Platelets	590	285	299	350	623
$\times 10^3$/mm^3	±46	±59	±41	±67	±80
	(5)	(5)	(6)	(6)	(6)
Factor VIII %	191	161	190	226	329
	±21	±23	±15	±24	±23
	(5)	(5)	(6)	(5)	(6)
Factor V %	415	269	801	1000	933
	±37	±53	±75	±85	±105
	(5)	(5)	(6)	(5)	(6)
Prothrombin	256	202	317	334	386
time %	±26	±35	±15	±25	±20
	(5)	(5)	(6)	(5)	(6)
Fibrinogen	165	180	370	483	508
mg/100 ml	±9	±16	±18	±48	±40
	(5)	(5)	(6)	(5)	(6)

Table III. Percent change four hours after provocative injection

	WBC	Platelets	Factor VIII	Factor V	Prothrombin time	Fibrinogen
Saline	+3	+28	+9	+59	+15	+38 (+133 mg/100 ml)
Endotoxin	—86	—39	—50	—60	—44	—49 (172mg/100 ml)

B. Effect of Varying the Provocative Dose of Endotoxin with the Preparative Amount Constant

Having established in the preceding experiments that 0.1 mg/kg of endotoxin constituted an adequate preparative dose, the effect of variation in the provocative dose at 24 hours was studied.

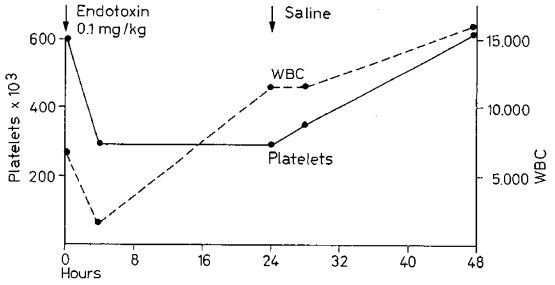

Fig. 3. Mean values for the white blood cells and platelets in rabbits receiving 0.100 mg/kg endotoxin for preparation and saline at 24 hours.

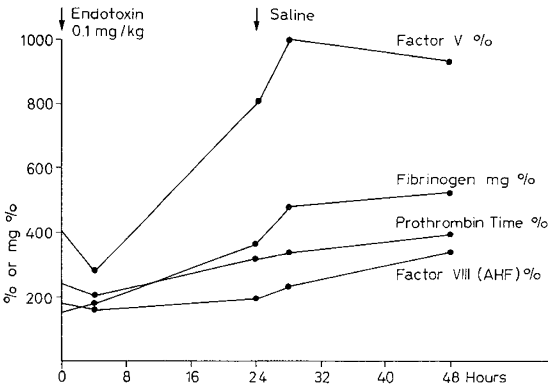

Fig. 4. Mean values for Factor V, Factor VIII, the prothrombin time and fibrinogen in animals prepared with 0.100 mg/kg endotoxin and saline at 24 hours.

All rabbits received 0.1 mg/kg endotoxin as the first injection. Twenty-four hours later the second injection, containing concentrations of endotoxin in decreasing amounts from 0.2 mg/kg to 0 (saline) was administered. Coagulation changes were measured four hours after each provocative dose and the results expressed in terms of percent change from the values at twenty-four hours. Since all rabbits received the same preparative dose, the coagulation data at twenty-four hours were combined for the entire group, as shown in table IV, and used as the baseline. The results of this experiment are given in table V. With 0.2, 0.1, 0.05 mg/kg provocation renal cortical necrosis was observed in 60 to 100 % of the animals. Rabbits given 0.01 mg/kg or saline did not develop the GSR. With provocative doses of 0.025 mg/kg and greater there was a significant fall in white cells, platelets and each of the coagulation factors measured. The fall in fibrinogen was significant at the 1 % level with doses of 0.2 and 0.1 mg/kg, at the 5 % level with 0.05 mg/kg and at the 10 % level with 0.025 mg/kg. With the provocative dose of 0.01 mg/kg there

Table IV. Values at 24 hours following preparation with 0.1 mg of *E. coli* endotoxin

	WBC (X 10^3/ mm^3)	Platelets (X 10^3/ mm^3)	Factor VIII (%)	Factor V (%)	Factor II (%)	Prothrombin time (%)	Fibrinogen (mg/ 100 ml)
Mean	11.1	272	207	627	165	289	350
Standard error	±6.5	±19	±12	±12	±22	±9	±13
Number of animals	38	47	51	49	25	49	51

Table V. Effect of varying provocative dose of endotoxin, preparative amount constant. The mean percent change four hours after the provocative injection and the incidence of the GSR are shown for the five groups of animals

Preparation (mg/kg)	0.1	0.1	0.1	0.1	0.1	0.1
Provocation (mg/kg)	Saline	0.010	0.025	0.05	0.1	0.2
Number of animals	10	10	12	11	15	10
WBC	+3	−28	−65	−80	−87	−86
Platelets	+28	0	−70	−79	−54	−39
Factor VIII	+9	−23	−38	−55	−51	−40
Factor V	+59	−54	−50	−45	−55	−60
Factor II	N.D.[1]	−34	−57	−71	N.D.	N.D.
Prothrombin time	+15	−21	−20	−47	−44	−41
Fibrinogen[2] (mg/100 ml)	+38 (+133)	−12 (−41)	−17 (−66)	−31 (−108)	−50 (−178)	−49 (−172)
GSR (renal necrosis)	0/20	0/19	10/17	7/7	10/13	34/49

[1] Not determined.

[2] Fibrinogen values are expressed as percent change and, in parentheses, in mg/100 ml.

was a significant decrease as Factors II, V and VIII but no significant fall in white cells, platelets or fibrinogen. The data with saline 'provocation' are in contrast to those observed with any of the endotoxin doses in that all of the measured factors rose as might have been anticipated from the data on fig. 3 and 4.

The changes in fibrinogen following the different provocative doses are demonstrated in more detail in fig. 5. The bars represent the mean and 95 %[3] confidence limits of the mean for the various fibrinogen levels. The number of animals in each experiment are shown in parentheses. At 28 hours, four hours following the provocative injection, fibrinogen increased to 482 mg/100 ml ±132 in the saline animals but declined to concentrations of 309 mg/100 ml ±53 following provocation with 0.010 mg/kg endotoxin, 284 mg/100 ml ±54 with 0.025 mg/kg endotoxin, 242 mg/100 ml ±56 with 0.05 mg/kg endotoxin, 172 mg/100 ml ±108 with 0.100 mg/kg endotoxin and 179 mg/100 ml ±45 with 0.200 mg/kg endotoxin.

Thus, all provocative doses of endotoxin tested resulted in a decrease in fibrinogen in the adequately prepared animal. However, renal cortical necrosis did not occur until a fall in fibrinogen of at least 66 mg/100 ml was produced.

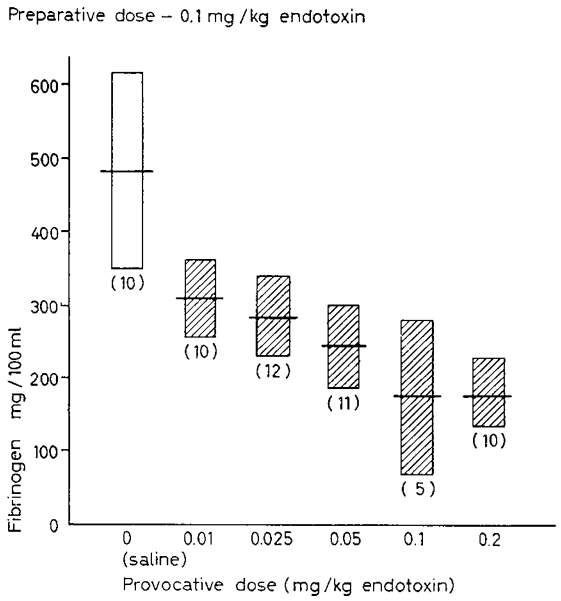

Fig. 5. Mean (horizontal line) and 95 % confidence limits (Bar) for fibrinogen four hours after provocation with various dose of endotoxin. All animals received 0.1 mg/kg endotoxin as preparation. The number of rabbits utilized in each experiment are shown in parathesis. A mean fibrinogen level of 350 mg/100 ml ±25 95 % CI was observed 24 hours after endotoxin preparation in 51 rabbits. Animals receiving saline at 24 hours demonstrated an increase in fibrinogen. In contrast all provocative doses of endotoxin produced a fall in fibrinogen which was directly related to the concentration of endotoxin employed.

[3] 95 % $CL\bar{y} = \bar{y} \pm (t \times s\bar{y})$.

Table VI. Effect of varying preparative dose of endotoxin, provocative amount constant. Mean percent change four hours after provocative injection and incidence of GSR are shown

Preparation (mg/kg)	Saline	0.001	0.01	0.1
Provocation (mg/kg)	0.1	0.1	0.1	0.1
Number of animals	10	10	15	25
WBC	—74	—72	—87	—87
Platelets	—41	—26	—66	—54
Factor VIII	—30	—1	—74	—51
Factor V	—14	—43	—48	—55
Factor II	—8	—41	—54	N.D.
Prothrombin time	—25	N.D.	N.D.	—44
Fibrinogen[4] (mg/100 ml)	—2 (—4)	—34 (—89)	—36 (—144)	—50 (—178)
Renal cortical necrosis	0/10	0/19	11/15	10/13

[4] Fibrinogen values are expressed as percent change and, in parentheses, in mg/100 ml.

2. Effect of Varying the Preparative Dose of Endotoxin

Four groups of rabbits were given either saline, 0.001 mg/kg, 0.01 mg/kg or 0.1 mg/kg endotoxin preparation and all animals received 0.1 mg/kg endotoxin as the second injection 24 hours later. The incidence of renal cortical necrosis is shown on table VI. With saline or 0.001 mg/kg endotoxin as preparation no GSR occurred in 10 and 19 animals respectively. However, with a 10 and 100 fold increase in the concentration of endotoxin the GSR was regularly produced. Similarly treated groups of rabbits were studied with respect to changes in the coagulation mechanism. Results are shown in table VI. With saline preparation the only significant change occurred in the white blood cells and platelets. However, a significant decline in all factors except Factor VIII[5] was produced with preparation by 0.001 mg/kg endotoxin but no GSR was produced. As was noted in the previous section a decline of as little as 66 mg/100 ml fibrinogen in the adequately prepared rabbit was sufficient to produce the GSR. With 0.001 mg/kg preparation the fibrinogen fell 89 mg/100 ml but no animal developed renal cortical necrosis. In other words, the inadequately prepared rabbit appeared able to tolerate an amount of fibrinogen conversion that would, in the properly prepared animal, result in renal cortical necrosis.

In the rabbits prepared with 0.01 and 0.1 mg/kg of endotoxin significant changes occurred in all factors and renal cortical necrosis was produced. Thus, 0.01 mg/kg of endotoxin constitutes adequate preparation when the dose of endotoxin for provocation is 0.1 mg/kg.

[5] Animals prepared with saline exhibited a 30 % fall in Factor VIII but only 1 % decline occurred in rabbits prepared with 0.001 mg/kg endotoxin. The experiment, however, demonstrated that 0.001 mg/kg endotoxin produced a higher level of Factor VIII at 24 hours than did 0.01 or 0.1 mg/kg endotoxin ('p' <0.01) thus suggesting that small doses of endotoxin stimulated Factor VIII production. It is our interpretation that Factor VIII utilization did indeed occur and that the lack of change four hours after provocation represents a resultant of stimulation by preparation and depletion by the provocative dose.

Table VII. Effect of interval between injections of endotoxin (Preparative dose 0.1 mg—provocative dose 0.1 mg)

	Mean % change four hours after provocative injection						
	WBC	Platelets	Factor VIII	Factor V	Prothrombin time	Fibrinogen	GSR
24 h (25)[6]	—87	—54	—51	—55	—44	—50 (—178 mg/100 ml)	10/13
48 h (18)[6]	—80	—70	—70	—41	—55	—18 (—70 mg/100 ml)	0/18

[6] No. of animals.

C. Effect of Interval Between Injections of Endotoxin

Rabbits were given 0.1 mg/kg of endotoxin as preparation. A provocative dose of 0.1 mg/kg was administered at 24 hours in one group of animals and 48 hours in a second group. In the 24-hour group the GSR occurred in 10 of 13 animals. Following endotoxin at 48 hours renal cortical necrosis was not produced in any of 18 animals. The changes that occurred in the coagulation mechanism four hours after provocation are shown in table VII. The mean percent fall was similar in both groups for all factors except fibrinogen. Although fibrinogen decreased 178 mg/100 ml in animals given two injections 24 hours apart there was only a 70 mg/100 ml fall in the 48-hour group of rabbits.

The data suggest that by 48 hours after adequate preparation the RES has recovered to the degree that

clotting intermediates are more effectively removed and that significant amounts of fibrin are effectively cleared, thus preventing deposition in the kidneys and the development of cortical necrosis.

D. Interdependence of Preparative and Provocative Dose of Endotoxin

Table VIII demonstrates that a dose of 0.01 mg/kg adequately prepares the animal for subsequent provocation with 0.1 mg/kg. Similarly, 0.1 mg/kg adequately prepares the animals for subsequent provocation with as little as 0.025 mg/kg. However, the combination of 0.01 mg/kg as preparation and 0.025 mg/kg as provocation does not result in cortical necrosis of the kidneys. The latter results are readily explainable when one measures the effect of these doses on the clotting system. Table IX demonstrates that even though the white blood cells and platelets declined four hours after the second injection the coagulation mechanism was not activated and there was no fall in any of the coagulation factors. Thus, 0.025 mg/kg of endotoxin was not sufficient, in the face of 0.01 mg/kg preparation, to induce intravascular clotting and ultimately the GSR.

Table VIII. Interdependence of preparative and provocative doses of endotoxin in production of renal cortical necrosis

Preparative dose (mg/kg)	Provocative dose (mg/kg)	Renal cortical necrosis
0.01	0.1	11/15 (74 %)
0.1	0.025	10/17 (60 %)
0.01	0.025	0/10 (0 %)

Table IX. Coagulation changes and incidence of GSR using minimal preparative and provocative amounts of endotoxin

Preparation—0.01 mg, Provocation = 0.025 mg	24 hours	28 hour	Percent change
Number of animals	5	5	–
WBC/mm³	12,850	5,720	—55
Platelets $\times 10^3$/mm³	433	341	—21
Factor VIII %	243	414	+70
Factor V %	1200	1000	—16
Factor II %	126	143	+13
Fibrinogen mg/100 ml	432	406	—6
Renal cortical necrosis		0/10	

Discussion

That the clotting mechanism is an integral part of the generalized Shwartzman reaction has been well documented [6, 9, 13, 23, 24]. The changes in the clotting factors following two injections of endotoxin in the typical GSR in this report are in agreement with the results reported by other investigators [13, 23, 24]. In addition our data demonstrate that in rabbits adequately prepared with endotoxin provocative doses leading to the consumption of as little as 66 mg/100 ml of fibrinogen results in the development of renal cortical necrosis.

However, the effects of variation of the preparative dose of endotoxin and of increasing the time interval between injections demonstrate that comparable changes in the measured clotting factors need not lead to bilateral renal cortical necrosis and suggest further that this may be related to the degree to which the RES remains capable of removing clotting intermediates and fibrin itself. The fact that disseminated intravascular clotting *per se* need not be followed uniformly by fibrin deposition in the kidneys is supported by the investigations by Lee who reported that prolonged intravascular clotting sustained by infusions of thrombin for 2 to 4 hours failed to produce the GSR in a significant number of animals [9]. The importance of the status of the RES is indicated by the fact that following blockade with Thorotrast or trypan blue the GSR can be elicited with a single intravenous injection of endotoxin, thrombin or immune complexes [9, 26, 27]. Endotoxin itself depresses RES function. Partial recovery from the effects of a single injection apparently occurs within 24 hours. A second injection at this time, however, induces marked impairment of clearing capacity [11, 31].

How endotoxin activates the clotting system is as yet unanswered. It has been suggested that endotoxin may act via two mechanisms, one by the activation of Factor XII (Hageman factor) directly [28] and the other through the release of platelet factor 3 [28, 29]. Although the data presented here do not bear directly on this problem it may be noted in table VI that even though there was a 41 % fall in platelets following a single injection of endotoxin significant utilization of plasma coagulation factors did not occur. This suggests that the destruction of platelets with the release of their clot promoting properties alone was not sufficient to activate the clotting system, *in vivo*.

Although the data in this report deal with the coagulation mechanism it should be noted that the concomitant association of an adequate number of white blood cells [15], an inefficient fibrinolytic mechanism [32, 33, 24], a sensitive vascular system [25, 30], and most notably an impaired RES [11] combined with

the changes in the clotting mechanism are essential for the production of the GSR.

In the investigations of patients with syndromes believed to represent the clinical counterpart of the GSR (purpura fulminans, the hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, the Waterhouse-Friderichsen syndrome, etc.) laboratory studies designed to detect the pattern of intravascular clotting are usually employed. In this regard the sequential changes observed in the endotoxin-induced GSR in the rabbit suggest that such laboratory determinations may not be definitive or diagnostic in individual cases even though intravascular clotting and fibrin deposition have indeed occurred. The data demonstrate that all clotting factors, with the exception of platelets, may return to normal levels very rapidly after a single insult capable of inducing marked intravascular clotting. Thus the time when blood samples are obtained may be critical in determining the pattern of values found. Furthermore the coagulation status of the individual *before* the provocative event may determine the absolute values found even though blood samples are obtained at the height of intravascular clotting. Thus, in the rabbit, the values for Factors V, VIII, and fibrinogen which were obtained four hours after the provocative injection, a time when fibrin deposition in the kidneys may be demonstrated pathologically, are not significantly different from those of entirely normal animals. However, as was shown, they are significantly lower than the values in the prepared animals immediately prior to provocation. It appears likely that these considerations explain, in part, the variety of coagulation results which have been reported in clinical situations [6, 35].

The observations of Good and Thomas which demonstrated that heparin, administered within four hours after the provoking dose of endotoxin, prevented the development of the GSR [36] have led to the use of heparin in human Shwartzman-like syndromes. Here, too, it would appear that the time of heparin administration as related to the status of intravascular clotting will determine the degree of benefit to be achieved. The studies in the rabbit indicate that intravascular clotting may occur as an abrupt and self-limited episode which is nevertheless capable of inducing severe pathological change. It seems evident that detailed and sequential coagulation studies will be required in the human syndromes before full understanding of their pathogenesis is achieved.

Studies by Robbins and Stetson [37, 38] have demonstrated that antigen-antibody reactions accelerate blood coagulation in vitro. Lee [26] has shown that in the presence of reticuloendothelial blockage injection of antigen into specifically immunized rabbits or the infusion of soluble antigen-antibody complexes in normal rabbits will lead to the development of renal cortical necrosis. The fact that cross-reactive antibodies to endotoxins have been demonstrated in rabbit sera [39], and that other similarities have been described between the effects produced by endotoxins and by antigen-antibody complexes [30, 41. 42] offers the possibility that endotoxin may exert its effect on intravascular clotting by virtue of its antigenicity. Thus the endotoxin-induced Shwartzman phenomenon may be but one example of a broad biologic phenomeon having its origin in antigen-antibody reactions and its expression in intravascular clotting.

Summary

1. Quantitative changes in coagulation Factors II, V, VIII, the prothrombin time, fibrinogen, platelets and the white blood cells were measured in rabbits given *E. coli* endotoxin.

2. In the presence of adequate preparation, provocative doses of endotoxin capable of inducing cortical necrosis of the kidneys were always associated with significant consumption of all of the coagulation factors measured.

3. With no preparation (saline) doses of endotoxin shown to be sufficient for provocation did not induce significant depletion of circulating plasma coagulation factors. With inadequate preparation, as produced either by decreasing the preparative dose of endotoxin or by increasing the time interval between injections, significant activation of the clotting system occurred but renal cortical necrosis did not develop.

4. The possible relationship of the experimental data to Shwartzman-like human syndromes was discussed.

References and Notes

1. Verstraete, M.; Vermylen, C.; Vermylen, J. and Vandenbroucke, J.: Excessive consumption of blood coagulation components as a cause of hemorrhagic diathesis. Amer. J. Med. *38:* 899 (1965).
2. McKay, D. G.: Diseases of hypersensitivity (Disseminated intravascular coagulation). Arch. intern. Med. *116:* 83 (1965).
3. Vassalli, P. and McCluskey, R. T.: The coagulation process and glomerular disease. Amer. J. Med. *39:* 179 (1965).
4. Rodriguez-Erdmann, F.: Bleeding due to increased intravascular blood coagulation. New Engl. J. Med. *273:* 1370 (1965).
5. Rosner, F. and Ritz, M. D.: The defibrination syndrome. Arch. intern. Med. *117:* 17 (1966).

6. HJORT, P.F. and RAPAPORT, S.I.: The Shwartzman reaction: pathogenetic mechanisms and clinical manifestations. Ann.Rev.Med. *16:* 135 (1965).
7. SMITH, R.T.; BRAUDE, A.I. and CAREY, F.J.: The distribution of Cr^{51} labeled *E. coli* endotoxin in the generalized Shwartzman reaction. J.clin.Invest. *36:* 695 (1957).
8. SPAET, T.H. and CINTRON, J.: Clearance of blood coagulation Product I in rabbits. Proc.Soc.exp. Biol. (N.Y.) *104:* 498 (1960).
9. LEE, L.: Reticuloendothelial clearance of circulating fibrin in the pathogenesis of the generalized Shwartzman reaction. J.exp.Med. *115:* 1065 (1965).
10. LEE, L. and MCCLUSKEY, R.T.: Immunohistochemical demonstration of the reticuloendothelial clearance of circulating fibrin aggregates. J.exp. Med. *116:* 611 (1962).
11. THOMAS, L.: The role of the reticuloendothelial system in the reaction to endotoxin; in HALPERN, B.N.; BENACERRAF, B. and DELAFRESNAYE, J.F.: Physiopathology of the reticulo-endothelial system, p.226 (Thomas, Springfield 1957).
12. GANS, H. and KRIVIT, W.: Effect of endotoxin on the clotting mechanism, Ann.Surg. *153:* 453 (1961).
13. MCKAY, D.G. and SHAPIRO, S.S.: Alterations in the blood coagulation system induced by bacterial endotoxin. I: *in vivo* (generalized Shwartzman reaction). J.exp.Med. *107:* 353 (1958).
14. APITZ, K.: A study of the generalized Shwartzman phenomenon. J.Immunol. *29:* 255 (1935).
15. THOMAS, L. and GOOD, R.A.: Studies on the generalized Shwartzman reaction. I.General observations concerning the phenomenon. J.exp. Med. *96:* 605 (1952).
16. BRUNSON, J.G.; GAMBLE, G.N. and THOMAS, L.: Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II.Two appropriately spaced injections: The role of fibrinoid in the GSR. Amer.J.Path. *31:* 655 (1955).
17. BRECHER, G. and CRONKITE, E.P.: Estimation of the number of platelets by phase microscopy; in TOCANTIUS, L.M.: The coagulation of blood: Methods of study; p.41 (Grune & Stratton, New York 1955).
18. QUICK, A.J.: Hemorrhagic Diseases; p.379 (Lea and Febiger, Philadelphia 1957).
19. OWEN, P.A. and AAS, K.: Control of dicumarol therapy and quantitative determination of prothrombin and proconvertin. Scand.J.clin.Lab. Invest. *3:* 201 (1951).
20. RATNOFF, O.E. and MENZIE, C.: New method for determination of fibrinogen in small samples of plasma. J.Lab.clin.Med. *37:* 316 (1951).
21. ABILDGAARD, C.F.; CORNET, J.A.; FORT, E. and SCHULMAN, I.: In vivo longevity of antihemophilic factor (Factor VIII). Brit.J.Haemat. *10:* 225 (1964).
22. BIGGS, R.P. and MACFARLANE, R.G.: Human blood coagulation and its disorder; 3rd Ed., p.388 (Blackwell Scientific Publications, Oxford).
23. KLEINMAIER, H.; GOERGEN, K.; LASCH, H.G.; KRECKE, H.J. und BOHLE, A.: Untersuchungen zur Frage der Gerinnungsstörung beim Sanarelli-Shwartzman-Phänomen (sog. generalisierten Shwartzman-Phänomen) des Kaninchens. Z. allg.Exp.Med. *132:* 275 (1959).
24. RODRIGUEZ-ERDMANN, F.: Studies on the pathogenesis of the generalized Shwartzman reaction. Throm.Diat.Haemorrh. *12:* 452 (1964).
25. GILBERT, R.P.: Mechanism of the hemodynamic effects of endotoxin. Physiol.Rev. *40:* 245 (1960).
26. LEE, L.: Antigen-antibody reaction in the pathogenesis of bilateral renal cortical necrosis. J.exp. Med. *117:* 365 (1963).
27. GOOD, R.A. and THOMAS, L.: Studies on the generalized Shwartzman phenomenon. II.The production of bilateral cortical necrosis of the kidneys by a single injection of bacterial toxin in rabbits previously treated with Thorotrast or trypan blue. J. exp.Med. *96:* 625 (1952).
28. RODRIGUEZ-ERDMANN, F.: III.Trigger mechanism for the activation of the prothrombin molecule. Throm.Diat.Haemorrh. *12:* 471 (1964).
29. HOROWITZ, H.I.: The effect of bacterial endotoxins on blood coagulation. Hémostase *4:* 43 (1964).
30. MCKAY, D.G. and MERRIAM, J.C.: Vascular changes induced by bacterial endotoxin during generalized Shwartzman reaction. Arch.Path. *69:* 524 (1960).
31. MCKAY, D.G.: A partial synthesis of the generalized Shwartzman reaction. Fed.Proc. *22:* 1373 (1963).
32. CONDIE, R.M.; HONG, C.Y. and GOOD, R.A.: Reversal of the lesions of the generalized Shwartzman phenomenon by treatment of rabbits with streptokinase. J.Lab.clin.Med. *50:* 803 (1957).
33. KLIMAN, A. and MCKAY, D.G.: The prevention of the generalized Shwartzman reaction by fibrinolytic activity. Arch.Path. *66:* 715 (1958).
34. RODRIGUEZ-ERDMANN, F.: Role played by experimentally induced fibrinolysis. Throm.Diath. Haemorrh. *12:* 462 (1964).
35. HJORT, P.F.; RAPAPORT, S.I. and JORGENSEN, L.: Purpura fulminans (Review). Scand.J.Haemat. *1:* 169 (1964).

36. GOOD, R.A. and THOMAS, L.: Studies on the generalized Shwartzman reaction. IV. Prevention of the local and generalized Shwartzman reactions with heparin. J. exp. Med. *97:* 871 (1953).
37. ROBBINS, J. and STETSON, C.A., Jr.: An effect of antigen-antibody interaction on blood coagulation. J. exp. Med. *109:* 1 (1959).
38. ROBBINS, J. and STETSON, C.A., Jr.: Mechanism of antigen-antibody reactions upon blood coagulation. Fed. Proc. *18:* 594 (1959).
39. LEE, L. and STETSON, C.A., Jr.: Studies on the mechanism of the Shwartzman phenomenon. Accelerated cutaneous reactivity to bacterial endotoxin. J. exp. Med. *111:* 761 (1960).
40. WEIL, M.H. and SPINK, W.W.: A comparison of shock due to endotoxin with anophylactic shock. J. Lab. clin. Med. *50: 511* (1957).
41. GILBERT, V.E. and BRANDE, A.I.: Reduction of serum complement in rabbits after injection of endotoxin. J. exp. Med. *116:* 477 (1962).
42. STETSON, C.A., Jr.: Symposium on bacterial endotoxins. IV. Immunological aspects of the host reaction endotoxins. Bact. Rev. *25:* 459 (1961).
43. The authors gratefully acknowledge the technical assistance of Mrs. Shirley Johnson, Miss Patt Simmons, and Mrs. Helen Gatton, and to Joseph V. Simone, M.D. for assistance and guidance in the statistical analysis of the data.
44. Presented in part at a meeting of the Society for Pediatric Research, Altantic City, New Jersey, April 30, 1966.
45. Supported by a graduate training grant (TI-AM-5344) from the National Institute of Arthritis and Metabolic Diseases, a grant (RG H-07418) from the National Heart Institute and a grant (RG HD-00568) from the National Institute of Child Health and Human Development, National Institutes of Health, United States Public Health Service.