EFFECTS OF BACTERIAL ENDOTOXIN ON RABBIT PLATELETS*

II. Enhancement of Platelet Factor 3 Activity in Vitro and in Vivo

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(Received for publication, June 4, 1962)

The occurrence of thrombocytopenia, platelet-leukocyte thrombi, intravascular deposition of fibrin, and thrombosis of small blood vessels during the evolution of the generalized Shwartzman reaction after spaced injections of bacterial endotoxins has directed attention to the effects of endotoxins on blood coagulation.

McKay, Shapiro, and Shanberge (1) demonstrated that incubation of whole blood or plasma with endotoxin resulted in a shortening of the clotting time in siliconized glassware due to an interaction of endotoxin and coagulation factors at some point prior to formation of blood thromboplastin. Robbins and Stetson (2) also observed that endotoxin accelerated coagulation *in vitro*, confirmed the suggestion of McKay and associates that platelets were necessary for this effect, and demonstrated that antigen-antibody reactions had entirely similar coagulation-accelerating effects *in vitro*. In addition, Robbins and Stetson (2) suggested that the endotoxin effect might be the result of an interaction between endotoxin and naturally occurring antibodies present in rabbit blood.

Previous studies from this laboratory have shown that incubation of platelet-rich rabbit plasma with endotoxin results in an increase in the activity of platelet factor 3, a thromboplastin precursor, and the transfer of serotonin and bactericidins against *Bacillus subtilis* from platelets to plasma (3). Platelet factor 3 is a platelet component which reacts with product I in the presence of factor V and calcium to form blood thromboplastin (4). The increased activity of platelet factor 3 provides at least one possible explanation of the *in vitro* effects of bacterial endotoxins on coagulation, but this observation does not exclude alterations in other coagulation factors.

The present studies have examined in detail the effects of endotoxin on blood coagulation *in vitro* and have failed to implicate participation of coagulation

* This work was supported by grants from the United States Public Health Service (Grants H3479, H5746, and A5515) and by the Health Research Council of The City of New York under Contract U-1107.

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factors other than platelet factor 3. Moreover, evidence has been obtained that a transfer of platelet factor 3 from platelets to plasma can be demonstrated *in vivo* after injection of endotoxin intravenously in rabbits.

Materials and Methods

Methods for Processing Blood Specimens.—All glassware was siliconized by immersion in a 1:1000 dilution of Dow Corning Z4141 for 1 hour and was baked for 2 hours at 170°C prior to use. Rabbit blood for *in vitro* experiments was obtained by cardiac puncture and was delivered through polyethylene tubing into one-tenth volume of 3.8 per cent sodium citrate solution. Blood for *in vivo* studies was obtained through polyethylene catheters previously positioned in the carotid artery. Catheters were throughly rinsed with saline solution between collections.

Platelet-rich plasma (PRP) was prepared by slow speed (100 *g*) centrifugation for 10 minutes and platelet-poor plasma (PPP) was obtained by centrifugation at high speed (39,000 *g*) for 30 minutes. PRP contained 300,000 to 600,000 platelets per mm^3 and PPP was free of platelets under phase microscopy. Blood and plasma samples were centrifuged at 4°C and were kept in an ice bath prior to use. In certain experiments, mixtures of PRP and endotoxin were incubated at 37°C in a standard bacteriology incubator or at 4°C in a refrigerator, and during incubation were constantly rotated at 15 RPM on a motor-driven Spinnerette (Model ST, New Brunswick Scientific Co., New Brunswick, New Jersey).

Reagents.—An *Escherichia coli* endotoxin of the Boivin type (Difco, Lot 0127:B8) suspended in saline solution to a concentration of 2 mg per ml was used in all experiments. Diatomaceous silica was obtained as filter-cel from Johns-Manville (Lampoc, California). Poliomyelitis immune globulin (Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York) was used as a source of human gamma globulin. Antiserum against human gamma globulin was prepared in rabbits by the Serology Section of the Central Laboratories, New York Hospital–Cornell Medical Center. The rabbit anti-human gamma globulin serum was of high titer and residual coagulant factors were inactivated by heating at 60°C for 5 minutes. Human thrombin (human plasma thrombin, crude, Cutter Laboratories, Berkeley) and human fibrinogen (Cutter Laboratories) were suspended in isotonic saline solution. Cephalin was prepared by the method of Bell and Alton (5). Stable product I was prepared from rabbit instead of human blood reagents according to the method of Zucker-Franklin, Javid, and Spaet (6).

Coagulation Studies.—The amount of platelet factor 3 activity available to the clotting process was determined by adding preformed product I and calcium to the test plasma and determining the clotting time of the mixture. The time required for coagulation is referred to as the product I substrate time (3).

Enumeration of platelets, the clotting time of whole blood in silicone-coated glassware, the one-stage prothrombin time (Quick), a modified prothrombin time (Ware and Stragnell), fibrinogen content, activity of factors V and VII, and the plasma components of thromboplastin generation were determined by methods previously reported (7, 8).

EXPERIMENTAL

The Effect of Endotoxin on Blood Coagulation in Vitro.—McKay and associates (1) and Robbins and Stetson (2) have demonstrated that bacterial endotoxin shortens the coagulation time of whole blood in siliconized tubes or citrated PRP after recalcification. Coagulation acceleration was considerably greater in PRP than PPP, indicating that platelets were involved in the reac-

tion. As shown in Table I, these observations were readily confirmed. Whole blood to which had been added endotoxin in a concentration of 100 μg per ml clotted in approximately one-third the time necessary for coagulation of blood to which saline was added. In addition, PRP containing endotoxin in a concentration of 100 μg per ml coagulated 135 seconds after recalcification, whereas coagulation of PRP without endotoxin required 215 seconds. There was no difference in the coagulation time after recalcification of PPP containing endotoxin or saline.

The observation that platelets are required for coagulation acceleration *in vitro* strongly suggests that endotoxins act on the first stage of coagulation;

TABLE I

Certain Effects of Endotoxin on Coagulation

Material added	Clotting time			
	Whole blood	Recalcified PRP	Recalcified PPP	Product I substrate time PRP*
	min.	*sec.*	*sec.*	*sec.*
Saline 0.1 ml	34	215	>360	23.7
Endotoxin 100 μg/ml	11	135	>360	9.9
Cephalin 1:200	12	110	109	10.0
Endotoxin 100 μg/ml and cephalin 1:200	12	117	112	10.0

* Assayed after 60 minutes' incubation at 37°C.

i.e., the generation of thromboplastin. This thesis is also supported by two additional observations: (*a*) Endotoxin in a concentration of 100 μg per ml incubated at 37°C with PPP for 1, 5, 15, 30, or 60 minutes does not accelerate coagulation after recalcification, as compared to control PPP (Table II), and accordingly does not demonstrate direct thromboplastic activity. (*b*) A thrombin-like effect of endotoxin is excluded by the observation that a mixture of fibrinogen (3 mg per ml) and endotoxin (100 μg per ml) failed to coagulate during the course of incubation at 37°C for 24 hours. McKay, Shapiro, and Shanberge (1) also concluded that endotoxins do not possess properties of preformed thromboplastin or thrombin, but act on the coagulation system prior to the combination of thromboplastin precursors.

Analysis of the Acceleration of Thromboplastin Generation Induced by Endotoxin.—Thromboplastin generation can be considered as involving two processes which interact at their completion. In one process, a number of coagulation factors (VIII, IX, X, XI, and XII) interact in the presence of calcium to form a soluble protein substance with potent coagulant activity termed product I by Bergsagel and Hougie (9). The formation of product I is accelerated by foreign

surfaces and by small amounts of thrombin (10). In the other process, blood platelets, which in the intact state do not exhibit procoagulant activity, are altered or damaged in such a way that platelet factor 3 becomes available for interaction with product I (10). Blood thromboplastin is formed when product I interacts with available platelet factor 3 in the presence of factor V and calcium.

The activity of platelet factor 3 available for the clotting process can be quantitated by the product I substrate time (see Materials and Methods). As shown in Table I, incubation of PRP with endotoxin greatly accelerates the product I substrate time. Addition of product I and calcium to PRP which had been incubated with endotoxin in a concentration of 100 μg per ml at

TABLE II

Absence of Surface-Activating Effect of Endotoxin

Time of incubation (37°C)	Clotting time after recalcification (siliconized glassware)		
	PPP + endotoxin 100 μg/ml	PPP + saline	PPP + saline + celite*
min.	*sec.*	*sec.*	*sec.*
1	182.4	185.0	65.1
5	180.7	177.0	59.9
15	128.0	115.9	58.2
30	111.8	106.3	50.6
60	106.3	105.2	51.7

* Diatomaceous silica, 5 mg per ml.

37°C for 1 hour resulted in clot formation in 9.9 seconds, whereas control plasma incubated with saline clotted in 23.7 seconds. Brain cephalin can be used as a substitute for platelet factor 3 in readily available form (5). Thus, it is of interest that cephalin accelerates the coagulation time of whole blood or recalcified plasma to a degree quite comparable to that produced by incubation of blood or PRP with endotoxin (Table I). The effects of cephalin and endotoxin are not additive.

Although the above findings are consistent with the concept that the coagulation-accelerating effect of endotoxin is mediated by platelet damage which increases the availability of platelet factor 3 for interaction with product I to form blood thromboplastin, these observations do not exclude the possibility that thromboplastin generation is accelerated in other ways as well. Other possible mechanisms include (*a*) acceleration of product I formation by endotoxin acting as a foreign surface, and (*b*) increase in the activity of other coagulation factors as an indirect effect of the interaction between endotoxin and platelets or plasma factors. These possibilities were investigated as follows:

(*a*). PPP prepared from a single bleeding was divided into three aliquots in siliconized glass tubes. Endotoxin in a concentration of 100 μg per ml of plasma was added to one tube and a control amount of saline was added to another. To the third specimen 5 mg per ml of diatomaceous silica, a substance known to effect maximum surface activation in coagulation systems (11), was added. The three specimens were incubated at 37°C, aliquots were removed at intervals, and the coagulation times after recalcification determined.

A moderate acceleration of the recalcification time was observed during the course of incubation (Table II) but no difference between the PPP containing endotoxin and the saline-treated control PPP was observed. In contrast, the effect of surface activation is illustrated by the appreciable coagulation ac-

TABLE III

Measurement of Other Coagulation Factors before and after Incubation of PRP or PPP with Endotoxin

Coagulation test	Factors measured	Clotting time after recalcification*			
		PRP		PPP	
		Endotoxin	Saline	Endotoxin	Saline
		sec.	*sec.*	*sec.*	*sec.*
Prothrombin time (Quick)	I, II, V, VII, X	6.4	6.7	6.5	6.3
Prothrombin time (Ware and Stragnell)	II, VII, X	14.5	15.7	15.4	14.3
Factor V	V	11.9	12.4	11.8	12.4
Factor VII	VII	18.0	18.6	18.7	18.4
Plasma components of thromboplastin generation	V, VIII	6.0	5.9	5.6	5.8

* Incubated 60 minutes at 37°C and then centrifuged.

celeration observed in the PPP containing diatomaceous silica. These findings make it unlikely that endotoxin has any direct surface-activating property on coagulation systems.

(*b*) PRP or PPP was incubated with endotoxin in a concentration of 100 μg per ml. Control plasmas were incubated with saline solution. After incubation at 37°C for 1 hour, the specimens were centrifuged (to remove platelets from PRP) and the supernatant plasmas were employed in the coagulation tests indicated in Table III.

It is seen that exposure of either PRP or PPP to endotoxin did not increase the activity of prothrombin, factor V, factor VII, or the plasma components of thromboplastin generation as compared to the saline-incubated controls.

Effects of Concentration of Endotoxin and Temperature of Incubation on Platelet Factor 3 Activity in Vitro.—In previous studies (3) it was shown that platelet aggregation and release of platelet 5-hydroxytryptamine or bacteri-

cidins against *Bacillus subtilis* were strikingly influenced by dose of endotoxin and temperature of incubation. Enhancement of platelet factor 3 activity by endotoxin is also dose- and temperature-dependent.

Aliquots of citrated PRP were mixed with endotoxin in concentrations of 0.1, 1.0, 10, and 100 μg per ml of plasma, and slowly rotated at 37°C. 0.1 ml aliquots of the endotoxin-plasma mixtures were removed immediately after addition of endotoxin and at varying intervals thereafter, and immediately added to 0.2 ml of a stable product I–calcium chloride preparation for determination of product I substrate time.

As shown in Table IV acceleration of the product I substrate time varied directly with the dose of endotoxin and the duration of incubation. As little as 1 μg per ml of endotoxin consistently produced a shortening of the product I

TABLE IV

Effect of Dose of Endotoxin on Product I Substrate Time of Rabbit PRP

Endotoxin	No. of animals	Time incubated			
		0 min.	30 min.	60 min.	120 min.
		Product I substrate time*			
μg/ml		*sec.*	*sec.*	*sec.*	*sec.*
0	10	27.7±4.2	26.1±3.9	23.7±4.2	22.8±4.5
0.1	4	26.0±3.6	24.4±3.8	22.2±2.4	17.5±1.6
1.0	4	26.0±3.6	22.0±2.8	19.1±2.8	14.7±1.9
10.0	6	27.1±3.7	21.3±2.9	16.5‡±2.3	12.4‡±1.6
100	10	27.8±4.2	15.9‡±2.7	9.9‡±2.2	10.6‡±1.8

* Mean ± 1 standard error.

‡ $P < 0.01$.

substrate time, and 100 μg per ml of endotoxin produced maximal shortening, equal in magnitude to that observed after addition of cephalin in optimal concentrations to entirely replace platelet factor 3.

Aliquots of citrated PRP were mixed with endotoxin in a concentration of 100 μg per ml and slowly rotated at 4°, 25°, and 37°C. Controls consisted of PRP incubated with saline solution at each temperature. One-tenth ml aliquots of the mixtures were removed immediately and at intervals during incubation for determination of platelet factor 3 activity by the product I substrate time. Each sample was placed in a water bath at 37°C for 1 minute prior to testing.

As shown in Table V, a significant decrease in the product I substrate time occurred by 30 minutes after addition of endotoxin to PRP incubated at 37°C, and 60 minutes of incubation produced a maximal effect. In contrast, in the mixtures incubated at 25°C, no significant decrease in product I substrate time was observed until 120 minutes after addition of endotoxin. No shortening

whatever of the product I substrate time was apparent in the endotoxin-PRP mixtures incubated at 4°C.

Activation of Platelet Factor 3 by Antigen-Antibody Reactions in Vitro.— Studies by Robbins and Stetson (2) demonstrated that the interaction of protein or polysaccharide antigens with specific antibody in rabbit blood produced a marked acceleration of coagulation *in vitro* and that platelets were required for this effect. The following experiment suggests that the effect of antigen-antibody interaction on coagulation results from activation of platelet factor 3.

A 1:50 dilution of heat-inactivated rabbit anti-human gamma globulin serum was added to rabbit PRP containing varying concentrations of human gamma globulin. The mixtures were slowly rotated at 37°C as in previous experiments. Aliquots of each mixture were removed immediately and at intervals during incubation, and the product I substrate times determined.

TABLE V

Effect of Temperature on Product I Substrate Time of Rabbit PRP Incubated with Endotoxin

Endotoxin	No. of animals	Temperature	Incubation time			
			0 min.	30 min.	60 min.	120 min.
			Product I substrate time*			
μg/ml		*°C*	*sec.*	*sec.*	*sec.*	*sec.*
0	10	37	27.7±4.2	26.1±3.9	23.7±4.2	22.8±4.5
100	10	37	27.8±4.2	15.9‡±2.7	9.9‡±2.2	10.6‡±1.8
0	3	25	26.6±4.2	26.5±4.1	25.1±4.2	20.8±2.3
100	3	25	26.6±4.2	25.7±4.2	21.6±2.6	15.0±1.3
0	5	4	25.9±3.3	25.6±3.6	24.5±3.9	21.8±3.7
100	5	4	26.0±3.3	25.2±3.5	23.6±3.5	21.3±2.8

* Mean ± 1 standard error.

‡ $P < 0.01$.

As shown in Table VI, the interaction of human gamma globulin and antibody induced an increase in platelet factor 3 activity comparable to that produced by bacterial endotoxin in previous experiments. With the dilution of antiserum held at 1:50, optimal activation occurred after 60 minutes' incubation with 0.1 or 1.0 μg per ml of gamma globulin. Larger concentrations of gamma globulin were less effective than smaller doses, suggesting a prozone phenomenon as described by Robbins and Stetson (2) in similar systems.

A comparable increase in platelet factor 3 activity was observed after addition of human gamma globulin to PRP from an animal previously immunized against human gamma globulin (Table VII).

The Effect of Endotoxin on Blood Coagulation and Platelet Factor 3 Activity

in Vivo.—McKay and Shapiro (12) demonstrated a marked decrease in whole blood clotting time in silicone 4 hours after injection of endotoxin in rabbits. The coagulation time had returned to normal values by 24 hours after injection.

TABLE VI

Effect of Antigen-Antibody Reaction on Product I Substrate Time of Rabbit PRP

Antigen HGG	Antibody (Coombs serum) dilution	No. of animals	Incubation time			
			0 min.	30 min.	60 min.	120 min.
			Product I substrate time*			
μg/ml			*sec.*	*sec.*	*sec.*	*sec.*
0	1:50	4	36.8±6.6	34.1±8.0	30.9±7.6	27.4±5.6
1.0	0	4	41.5±6.6	36.5±3.9	30.6±2.6	28.3±1.6
0.1	1:50	4	34.3±6.0	15.9‡±2.1	13.8‡±0.9	14.8‡±0.5
1.0	1:50	4	34.7±6.6	16.4±2.8	13.2‡±1.4	14.3‡±1.5
10.0	1:50	4	39.4±7.4	24.1±6.1	19.2±3.7	19.0±3.6
100.0	1:50	4	37.7±6.3	30.2±5.2	23.7±3.4	22.0±1.5

* Mean ± standard error.

‡ $P < 0.01$.

TABLE VII

*Effect of Antigen on Product I Substrate Time of PRP from Immunized Rabbit**

Human gamma globulin	Incubation time					
	0 min.	15 min.	30 min.	45 min.	60 min.	90 min.
	Product I substrate time					
μg/ml	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
0	33.3	33.8	30.2	27.7	25.6	21.1
5	33.1	21.7	18.0	17.1	16.7	13.0
50	28.8	18.6	17.7	13.2	14.0	14.2
500	30.2	20.2	15.1	14.7	14.0	14.9

* Rabbit received biweekly subcutaneous injections of human gamma globulin in adjuvant for 5 weeks prior to testing.

In the following studies blood coagulation was investigated during the 1st hour after injection of endotoxin in rabbits.

Rabbits were prepared by insertion of an indwelling polyethylene catheter in the carotid artery. After recovery from anesthesia, baseline specimens of blood were obtained, and saline or endotoxin in doses of 0.5, 1.0, or 5.0 mg was injected *via* the marginal ear vein. Serial specimens were withdrawn through the intra-arterial catheter at 1, 5, 15, 30, and 60 minutes after injection. PRP and PPP were obtained as previously mentioned, and all specimens

were kept at 4°C prior to testing. Each sample was assayed for whole blood clotting time (silicone), platelet count, prothrombin time (Quick, and Ware and Stragnell methods), fibrinogen content, and the product I substrate time using both PRP and PPP.

The data from representative individual experiments are presented in Figs. 1 to 4. The magnitude of the changes observed in repeated individual experi-

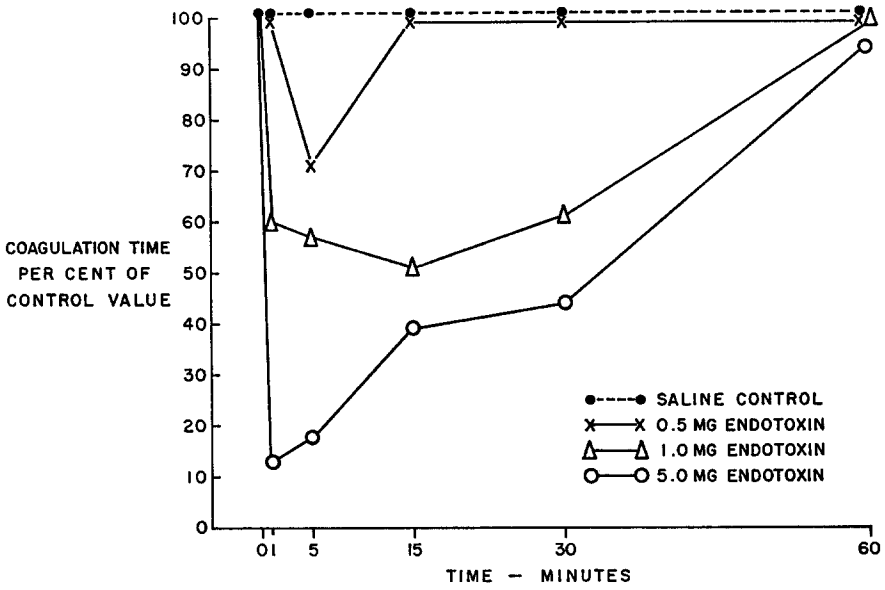

FIG. 1. Changes in coagulation time after intravenous injection of endotoxin in graded doses into rabbits. Changes in this and subsequent figures are expressed as per cent of pre-injection values.

ments varied widely, but were consistent in direction. A significant shortening of the clotting time was observed at the higher dose level, was most pronounced at 1 to 5 minutes after injection, and returned to the control value after 1 hour (Fig. 1). Thrombocytopenia occurred at all dosage levels (Fig. 2). After injection of 5.0 mg of endotoxin, platelets decreased to almost zero by 5 or 15 minutes and were still depressed at 60 minutes after injection. Platelet factor 3 activity as measured by the product I time with PRP substrate was also maximally increased in the earliest specimens and returned to control values by 1 hour (Fig. 3).

The finding of accelerated coagulation with increased platelet factor 3 activity concurrent with marked thrombocytopenia can be interpreted as indicating either that the few remaining platelets were more active than normal platelets or that platelet factor 3 had been released from aggregated and sequestered platelets and was present in plasma. The changes in product I

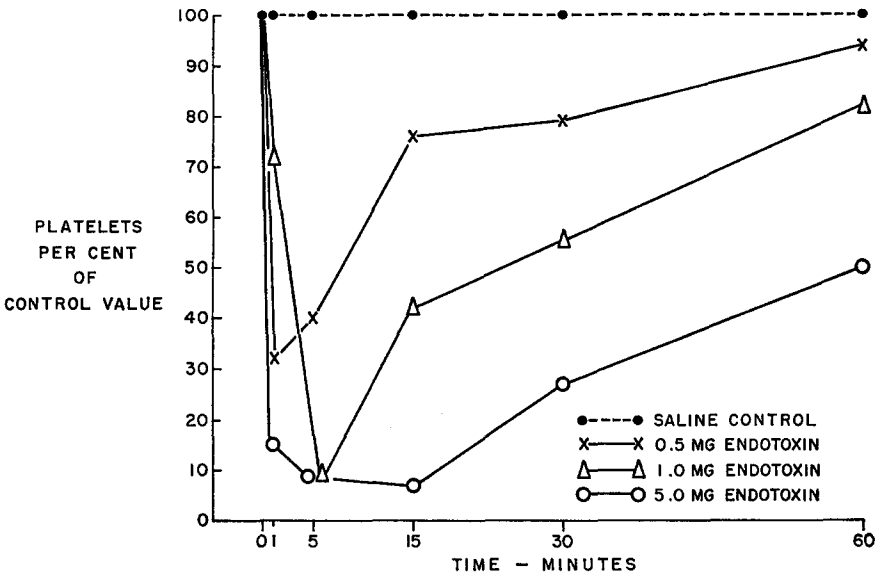

FIG. 2. Changes in platelet count after intravenous injection of endotoxin in graded doses into rabbits.

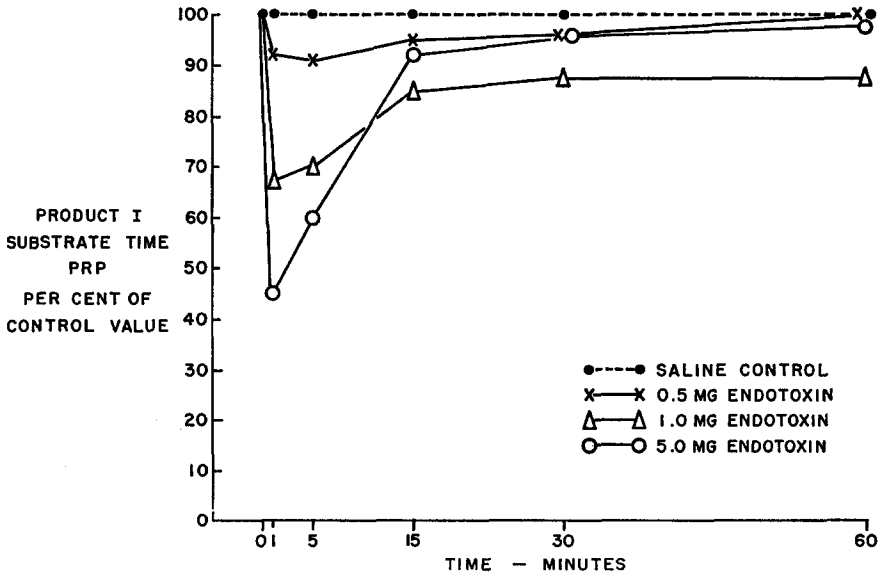

FIG. 3. Changes in product I substrate time of PRP after intravenous injection of endotoxin in graded doses into rabbits.

time with PPP substrate indicate that the latter appears to be the case (Fig. 4). These changes closely parallel the findings with PRP, indicating that platelet factor 3 is transiently present in plasma apart from platelets following injection of endotoxin.

No change was noted in fibrinogen concentration or in either of the prothrombin tests, indicating that appreciable intravascular coagulation did not occur.

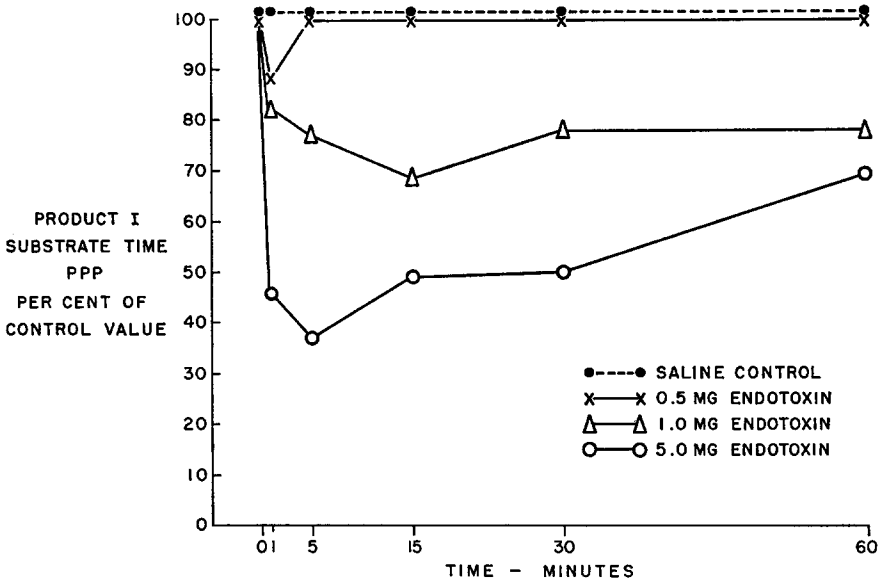

FIG. 4. Changes in product I substrate time of PPP after intravenous injection of endotoxin in graded doses into rabbits.

DISCUSSION

Previous studies (3) have shown that bacterial endotoxins produce platelet aggregation and release of certain platelet factors. Platelet factor 3, a thromboplastin precursor, is present in platelets in a form not readily available to the clotting process (10, 13). The present studies, utilizing the product I substrate time as an assay of platelet factor 3 activity, suggest that endotoxin by an action on blood platelets *in vitro* or *in vivo* increases the availability of platelet factor 3 for thromboplastin formation. Moreover, detailed analysis of the effects of endotoxin on the coagulation mechanism *in vitro* has failed to demonstrate significant alteration in other coagulation factors. It seems likely that the action of endotoxin on platelets increasing the availability of platelet factor 3 is at least one mechanism by which endotoxins accelerate blood coagulation.

This concept is further supported by observations that a comparable acceleration of coagulation can be achieved *in vitro* by thermal or mechanical disruption of platelets to increase the availability of platelet factor 3 (10, 13, 14), or *in vivo* by injection of phosphatides with platelet factor 3 activity (15, 16).

Changes in blood coagulation after injection of endotoxin may play a crucial role in the pathogenesis of the generalized Shwartzman phenomenon (GSR). The GSR is classically produced by injection of two sublethal doses of endotoxin in rabbits within a 24 hour period and is characterized by widespread intravascular deposition of fibrinoid material particularly in glomerular capillaries and by bilateral renal cortical necrosis (17). The fibrinoid material appears to be derived from fibrinogen, resembles fibrin in many respects, and may represent fibrin in a stage of partial polymerization (18). Although the initial or preparatory injection of endotoxin in rabbits apparently results in an increase in the level of fibrinogen by 24 hours after injection, the second or provocative injection of endotoxin given 18 to 24 hours after the first is followed by marked decrease in circulating fibrinogen (12). Defibrination and deposition of intravascular fibrinoid material occur at about the same time after injection of endotoxin, consistent with a cause and effect relationship (12). The observations that certain anticoagulants including heparin (19) and sodium warfarin (20) prevent deposition of intravascular fibrinoid also implicate blood coagulation in the genesis of the GSR.

Any explanation of the mechanism of the GSR must account for the requirement of two injections of endotoxin spaced about 24 hours apart. Thomas has cited evidence that the first dose of endotoxin impairs the function of the reticuloendothelial system, and has postulated that this impairment compromises the capacity to detoxify the second dose, which then circulates longer and reaches different tissue sites than the first dose (18). This hypothesis is consistent with the observation that the GSR can be produced after a single dose of endotoxin in animals pretreated with thorotrast, trypan blue, colloidal iron or carbon, or cortisone, all of which substances may impair reticuloendothelial function (18, 21, 22).

A recent report by Lee has offered an hypothesis which integrates altered coagulation and depressed reticuloendothelial function in the pathogenesis of the GSR (23). Previous workers had noted that intravenous infusion of large doses of thromboplastin or thrombin, although initiating intravascular coagulation, did not produce lesions typical of the GSR (24–26). In these experiments thrombi appeared predominantly in the lungs and veins but not in the kidneys. Lee reasoned that an efficient mechanism exists for removing products of fibrin polymerization from the general circulation, and investigated the possibilities of activation of proteolytic enzymes with fibrinolytic activity, and removal of fibrin or fibrin intermediates from the circulation by reticuloendothelial cells. Evidence of an active fibrinolytic mechanism in rabbits after injection of endotoxin or thrombin could not be obtained. However, it was possible to demonstrate that thrombin, in doses which only occasionally produced lesions of the GSR in normal animals, regularly induced renal cortical necrosis and changes typical of the GSR in rabbits in which reticuloendothelial function had been depressed by prior injections of thorotrast, denatured albumin, or endotoxin. In these studies it was also observed that thorotrast given as long as 3 hours after endotoxin increased the incidence of the GSR, indicating that the thorotrast effect was directed at intravas-

cular events occurring subsequent to the disappearance of endotoxin from the circulation. As an explanation of these observations Lee proposed that the reticuloendothelial system of normal animals functions efficiently to remove fibrin polymers formed in the blood stream, that the first dose of endotoxin given to prepare for the GSR serves to depress reticuloendothelial function, and that fibrin formed after the second injection of endotoxin is accordingly not cleared from the blood stream and is deposited as fibrinoid in terminal vascular beds.

Whether the reticuloendothelial system plays a major role in the removal of fibrin or fibrinogen polymers from the blood stream has not yet been determined. However, recent observations by Spaet and associates (27) indicate that thromboplastin and thromboplastin precursors are cleared by the reticuloendothelial system with sufficient avidity that injections of rather large quantities of these materials do not result in intravascular coagulation. These authors believe that the clearance of clotting intermediates by the reticuloendothelial system is an important factor in the preservation of blood fluidity *in vivo*. Thus, in states characterized by alterations in the rate of formation or activation of thromboplastin or thromboplastin precursors the normal reticuloendothelial system is competent to remove the procoagulant substance and prevent thrombin formation and conversion of fibrinogen to fibrin. In this regard, Adelson and associates (28) have demonstrated that hypercoagulable states accompanying thrombus formation, operative procedures, and epinephrine injection considerably modify the half-life of labeled platelets but have no effect on the rate of utilization of labeled fibrinogen.

The rapid disappearance of platelet factor 3 activity from platelet-poor plasma of rabbits after injection of a single dose of endotoxin as demonstrated in the present studies raises the possibility that this factor, like thromboplastin, is removed from the blood stream by the reticuloendothelial system. If this is correct, depression of reticuloendothelial function might result in accumulation of thromboplastin in amounts sufficient to initiate widespread intravascular coagulation. It is possible that in the pathogenesis of the GSR altered clearance by the reticuloendothelial system of thromboplastin or thromboplastin precursors is as important as the altered clearance of fibrin postulated by Lee (23).

The changes in activity of platelet factor 3 appear to be an adequate explanation for the changes in coagulation observed during the 1st hour after injection of endotoxin in rabbits, but are not necessarily related to the coagulation acceleration noted by McKay and Shapiro 4 hours after endotoxin injections (12).

SUMMARY

Incubation of rabbit platelet-rich plasma with bacterial endotoxin results in activation of platelet factor 3, a precursor of blood thromboplastin. This platelet-endotoxin interaction is dose- and temperature-dependent, and is similar to the effects of antigen-antibody union in the presence of platelets. Injection of endotoxin intravenously is associated with a transient increase in

platelet factor 3. Since platelet factor 3 can be demonstrated in platelet-poor plasma as well as platelet-rich plasma after endotoxin injection, it seems likely that an actual transfer of factor 3 from platelets to plasma occurs and that this activity is then rapidly inactivated or cleared from the blood stream.

The assistance of Miss Mitzu Fujimoto and Miss Aki Yamamoto is gratefully acknowledged.

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