RETICULOENDOTHELIAL CLEARANCE OF CIRCULATING FIBRIN IN THE PATHOGENESIS OF THE GENERALIZED SHWARTZMAN REACTION*

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Abundant evidence has accumulated during recent years to indicate that intravascular coagulation may be the determining event in the pathogenesis of the generalized Shwartzman reaction.

The renal cortical necrosis, which occurs after the second of two intravenous injections of bacterial endotoxin, has been shown to be due to occlusion of glomerular capillaries by material resembling fibrin (1–4). Deposition of this material can be blocked by the administration of heparin (5) or coumarin compounds (6) in doses rendering the blood incoagulable. Administration of streptokinase, in doses that have been demonstrated to produce *in vivo* fibrinolysis, also prevents the accumulation of these intravascular deposits (7). Finally, endotoxin has been shown to promote blood clotting both *in vitro* (8) and *in vivo* (9) with a demonstrable fall in fibrinogen level concurrent with the appearance of "fibrinoid" or fibrin deposits in the glomerular capillaries.

Despite the evidence implicating the coagulation mechanism in the pathogenesis of this phenomenon, infusions of thrombin or thromboplastin (10–13) have not produced lesions resembling those of the generalized Shwartzman reaction, with a single exception: Robbins and Collins (14) have shown that the administration of thrombin directly into the renal arterial circulation does indeed result in the appearance of classical bilateral renal cortical necrosis associated with massive fibrin deposition in glomerular capillaries. The success of this experiment, as contrasted with the negative results obtained when thrombin is infused intravenously, suggests the hypothesis that an efficient mechanism may exist for removing the products of fibrin polymerization formed in the general circulation. The present study has provided evidence indicating that in the rabbit the reticuloendothelial system may be the mechanism responsible for the clearance of circulating fibrin polymers and in this report experiments are described further elucidating the role of intravascular

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clotting and of reticuloendothelial function in the production of bilateral renal cortical necrosis.

Materials and Methods

Animals.—Albino hybrid rabbits weighing approximately 1.2 to 1.6 kilos were used.

Endotoxin.—Lyophilized lipopolysaccharide of *Escherichia coli* 0111:B4 (Difco Laboratories, Inc., Detroit) was dissolved in physiologic saline solution, and 2.0 ml volumes were used for intravenous injections.

Thorotrast.—A sterile, stabilized colloidal suspension containing 24 to 26 per cent thorium dioxide was obtained from Testagar & Co., Inc., Detroit. The intravenous dose used for reticuloendothelial blockade was 3.0 ml per kilo of body weight.

Intravenous Infusions.—Test materials were diluted to desired concentrations for intravenous infusion in bottles of sterile, pyrogen-free, physiologic saline solution. Rabbits were placed in individual stalls with their heads immobilized, and solutions were slowly infused into the marginal ear veins using ordinary sterile parenteral administration sets. The approximate rate of flow was determined by observing the number of drops per minute in the drip chamber.

Thrombin, Topical (Parke-Davis & Co., Detroit) was a sterile commercial preparation of bovine origin containing 5000 N.I.H. units per vial. The lyophilized material was freshly reconstituted and diluted with physiologic saline to the desired final concentrations just prior to intravenous administration. *Epsilon-aminocaproic acid* (Lederle Laboratories, Pearl River, New York) for parenteral injections was obtained in solutions containing 250 mg per ml. *Heat denatured albumin* in colloidal suspension was prepared from a 1 per cent solution of bovine albumin, fraction V powder (Pentex, Inc., Kankakee, Ill.) in physiologic saline, denatured by the method of Benacerraf *et al.* (15).

Assays of Plasma for Proteolytic and Fibrinolytic Activity.—Assays were performed on the euglobulin fraction obtained by precipitation at pH 5.2 from plasma diluted 1 to 20 with distilled water. *Proteolytic activity* was determined by the digestion of casein using the method of Mullertz (16). *Fibrinolytic activity* was estimated by the standard fibrin plate method of Astrup and Mullertz (17), and by determination of the lysis time of sterile whole blood clots and of euglobulin clots.

Carbon Clearance Studies.—The phagocytic activity of the reticuloendothelial system was measured by the clearance of carbon particles from the blood stream according to the method of Biozzi, Benacerraf, and Halpern (18). A standard dose of 4 mg carbon/100 gm body weight was injected intravenously. The rate of clearance expressed by the phagocytic index K was calculated from the equation: $(\log C_1 - \log C_2)/(T_1 - T_2) = K$, where C_1 and C_2 are the concentrations of carbon measured respectively at times T_1 and T_2.

Histologic Studies.—Autopsies were performed immediately following sacrifice of the animals by cervical dislocation, except in cases in which the rabbits died during the night. Samples of the visceral organs were fixed in 10 per cent neutral formalin, and sections were stained with hematoxylin-eosin, periodic acid-Schiff, and phosphotungstic acid-hematoxylin.

EXPERIMENTAL

Effect of Sublethal Intravenous Infusions of Thrombin in Normal Rabbits.—

35 normal rabbits received intravenous infusions of a dilute thrombin solution (2 to 3 units/ml) *via* the marginal ear veins over an interval of 2 to 4 hours. When thrombin was administered at a rate not exceeding 2.5 units per minute, 90 per cent of the animals survived the experiment. The total amount of thrombin infused ranged from 250 to 600 units for each rab-

bit. The survivors were sacrificed 24 hours later; the pathologic findings are presented in Table I.

In accordance with results of previous investigations (10–13), it was found that most of these animals exhibited no gross or histologic evidence of renal cortical necrosis. However, 4 of the 35 rabbits did show morphologic alterations considered pathognomonic of the generalized Shwartzman reaction (1, 2). The kidneys (Fig. 1) appeared markedly swollen with a variegated surface pattern produced by numerous necrotic and hemorrhagic foci which were predominantly cortical in distribution with encroachment on the medulla in the fully developed lesion. The histologic picture (Fig. 2) was characterized by disruption of the normal architecture of the cortex and subjacent medulla by interstitial hemorrhage and confluent zones of tubular necrosis. In almost every glomerular tuft dense, amorphous fibrin deposits occluded or narrowed the capillary

TABLE I

Incidence of Bilateral Renal Cortical Necrosis and Fibrin Deposition after a Sublethal Infusion of Thrombin

Dose of thrombin	No. of rabbits	No. with bilateral renal cortical necrosis	No. with microscopic fibrin deposits in	
			Kidney	Other organs
units				
250 to 300	17	2	3	4
300 to 400	10	1	2	3
400 to 600	8	1	1	3

lumina. By morphologic evaluation or histochemical reactions these fibrin masses appeared identical to the classical "fibrinoid" of the generalized Shwartzman reaction. In these four animals intravascular fibrin deposition, hemorrhage, and necrosis were also found in the liver, spleen, and lungs, but these findings were less conspicuous and were irregular in occurrence.

Of the 31 rabbits whose kidneys were normal in the gross at autopsy, two revealed patchy fibrin deposits in the glomeruli on microscopic examination. In addition, in approximately 20 per cent of the animals histologic inspection of the liver, spleen, and lungs showed lesions which were sparsely distributed but were morphologically similar to the findings in those rabbits exhibiting renal cortical necrosis.

These observations clearly indicated that prolonged intravascular clotting sustained by continuous infusions of thrombin could duplicate all of the pathologic features usually associated with the generalized Shwartzman phenomenon. However, only a few animals developed renal cortical necrosis with massive fibrin deposition, and no correlation was found between the incidence of the

renal lesion and the quantity of thrombin administered (Table I). In experimental situations in which a cause and its effect are being studied, the lack of a good dose-response relationship is usually interpreted as indicating either (*a*) that the response is not a direct effect of the administered material, or (*b*) that a protective or inhibitory mechanism exists. In this instance, it seemed most likely that the response (formation and deposition of fibrin) was in fact a direct effect of the agent (thrombin) administered. Therefore, the more reasonable interpretation of these findings is that rabbits possess some mechanism which protects in varying degrees against intravascular fibrin formation or deposition.

Incidence of Renal Cortical Necrosis and Fibrin Deposition in Glomeruli after a Single Intravenous Injection of Endotoxin.—The results described above have a significant parallel in the findings in rabbits subjected to a single intravenous

TABLE II

Incidence of Renal Cortical Necrosis and Fibrin Deposition in Glomeruli After a Single Injection of Endotoxin

No. of rabbits	No. with fibrin in glomeruli	No. with renal cortical necrosis	
		Microscopic	Macroscopic
Dead within 24 hours, 26	11 (42 per cent)	5 (19 per cent)	0
Alive, sacrificed at 24 hours, 74	0	0	0
Total, 100	11 (11 per cent)	5 (5 per cent)	0

injection of endotoxin, in which situation the deposition of fibrin in the peripheral vascular bed is usually absent or minimal, with only rare animals showing lesions approaching in severity and extent to those of the generalized Shwartzman reaction (19). During the present study an opportunity to confirm these findings was at hand.

Over a 6 month period, one hundred normal rabbits received single intravenous injections of 0.4 mg of endotoxin. Those animals surviving to the 24th hour were sacrificed, and autopsies were performed on all rabbits. The results of examination of the kidneys of these animals are summarized in Table II.

The significant feature of this survey was the finding that fibrin accumulation in glomeruli occurred only in those rabbits which had succumbed to the endotoxin, and in several of these early renal tubular necrosis was observed on microscopic examination. The unexpectedly high incidence of intravascular fibrin deposition in dying animals suggests as a possibility a general failure of homeostasis pertaining not only to the lethal action of endotoxin but also to the removal of the products of intravascular coagulation.

There can be no doubt, in view of previous studies (10, 13, 20, 21), that the

intravenous administration of sublethal doses of thrombin or thromboplastin results in the intravascular conversion of substantial amounts of the animal's fibrinogen to fibrin, and there is good evidence (3–9) that similar intravascular fibrin formation occurs following administration of endotoxin. Some mechanism must then exist for the removal of circulating fibrin polymers, since it usually cannot be found deposited in appreciable quantities in any of the tissues of the animal. Two chief possibilities immediately suggested themselves: (*a*) that injections of thrombin or endotoxin in the rabbit, in addition to initiating intravascular clotting, might cause activation of plasminogen or other plasma proteolytic enzymes, with resulting fibrinolysis as has been shown in man (22); or (*b*) that aggregates of fibrin or of fibrin intermediates may be efficiently removed from the circulation by the phagocytic activity of the reticuloendothelial system. In the next section the first of these possibilities is considered, and the results of attempts to demonstrate fibrinolysis in rabbits following injections of endotoxin or thrombin are presented.

Fibrinolysis in the Generalized Shwartzman Reaction.—

A. Effect of EACA Administration on the Occurrence of Renal Cortical Necrosis: The existence of a potentially effective fibrinolytic system in the rabbit was demonstrated by Condie *et al.* (7) who showed that the administration of streptokinase prevented the lesions of the generalized Shwartzman reaction. In order to test the possibility that intravascular fibrinolysis might be responsible for the failure of accumulation of fibrin in animals receiving thrombin or endotoxin, advantage was taken of the recent finding that ε-aminocaproic acid (EACA) is a relatively specific and effective inhibitor *in vivo* (23) and *in vitro* (24) of the activation of plasminogen. The suitability of this material for use in the present study is predicated on the assumption that it has no other biological activity relevant to this experimental model. While adequate information along these lines is not yet available, the studies of Zweifach *et al.* (25) have shown at least that EACA produces no significant alterations in vasomotor activity, coagulation, or peripheral white blood cell count. In the next experiment, EACA was given in conjunction with a single injection of endotoxin in the expectation that inhibition of fibrinolysis by EACA might permit the development of the generalized Shwartzman reaction.

Endotoxin and EACA.

Four groups of rabbits received single intravenous injections of 0.4 mg of endotoxin. Either simultaneously or at 1, 3, and 5 hours after the endotoxin injections, continuous intravenous infusions of EACA were started in each group. A total quantity of 1.5 gm of EACA diluted in 100 ml of physiologic saline solution was given to each rabbit over an interval of 2½ to 3 hours. As controls, two groups of rabbits received intravenous infusions of either saline or L-lysine in equivalent volume, quantity, and rate of administration. In addition, the effect of EACA infusion in normal rabbits was examined. Lastly, two groups of animals received rapid single intravenous injections of 1.5 gm of EACA (6 ml) within 30 seconds, and then either at the same time or at 3 hours later an intravenous injection of 0.4 mg of endotoxin

was given to each rabbit. All survivors were sacrificed and autopsied 24 hours after the start of the experiment.

Thomas and Good (1) had observed gross renal cortical necrosis in only two rabbits in a series of 265 receiving single intravenous injections of endotoxin. However, it can be seen from Table III that a single injection of endotoxin followed by an infusion of EACA either immediately or even as late as 3 hours afterward resulted in a high incidence of typical renal cortical necrosis. The gross appearance as well as the histopathology of the kidneys was identical to the severe lesions elicited by the classical procedure of two intravenous injections of endotoxin (Figs. 1 and 2). Lysine, differing from EACA in having

TABLE III

Effect of EACA Administration on the Production of Bilateral Renal Cortical Necrosis by Endotoxin

Experimental procedure	Time interval	No. of rabbits	No. with bilateral renal cortical necrosis
	hours		
Endotoxin followed by continuous EACA infusion	0	5	3
	1	4	2
	3	5	3
	5	4	0
Single rapid intravenous injection of EACA followed by endotoxin	0	4	0
	3	4	0
EACA infusion alone	—	8	0
Endotoxin followed by lysine infusion	0	4	0
Endotoxin followed by saline infusion	0	8	0

an α-NH_2 group, was found by Mullertz (26) to have only a weak antifibrinolytic activity *in vitro* and in this experiment was ineffective as a substitute for EACA. The action of EACA appeared to be transitory, as indicated by its inefficacy when administered as a single rapid intravenous injection even in amounts equivalent to the total quantity infused over a 2 to 3 hour period. The continuous infusion of EACA in normal rabbits produced no morphologic changes in the visceral organs, nor did injections of endotoxin alone result in renal cortical necrosis.

The synergistic role of EACA in these experiments was probably not in the enhancement of any direct effect of endotoxin. This was shown by the high incidence of renal cortical necrosis when EACA was given as late as 3 hours after an endotoxin injection, at a time when the endotoxin had long since disappeared from the blood stream (27). On the other hand, the findings were

consistent with the concept that a single intravenous injection of endotoxin is capable of producing a prolonged period of continuous intravascular coagulation, and provided support for the proposal that the usual failure of fibrin accumulation after a single injection of endotoxin is due to its rapid removal by a fibrinolytic mechanism.

Intravenous Thrombin Infusion and EACA.—The thrombin infusion model offered an opportunity to study another biological system in which intravascular coagulation and fibrin deposition are known variables.

One group of rabbits received simultaneous infusions of thrombin and EACA separately in opposite ear veins. The other groups received intravenous infusions of thrombin alone or EACA alone. A total of 300 to 350 units of thrombin and 0.5–1.0 gm of EACA were administered over a two hour interval. The results are presented in Table IV.

TABLE IV

Effect of EACA on the Production of Bilateral Renal Cortical Necrosis by Thrombin Infusion

Experimental procedure	No. of rabbits	No. with bilateral renal cortical necrosis
Simultaneous infusion of thrombin and EACA	7	5
Infusion of thrombin alone	9	1
Infusion of EACA alone	8	0

It was evident that the administration of EACA concurrent with the infusion of thrombin promoted massive fibrin deposition and greatly increased the incidence of renal cortical necrosis. Both in rabbits treated with endotoxin or infused with thrombin, then, the results obtained with EACA were consistent with the existence of a potent fibrinolytic mechanism.

B. In Vitro Assays for Proteolytic and Fibrinolytic Activity: The next experiments were designed to secure direct evidence for the existence of increased proteolytic or fibrinolytic activity in the plasma of rabbits given a single injection of endotoxin or an intravenous infusion of thrombin.

Six rabbits received single intravenous injections of 0.2 mg of endotoxin; blood specimens were obtained by cardiac puncture before and at 1, 2, and 4 hours after the endotoxin injection and were tested for spontaneous proteolytic or fibrinolytic activity. 3 additional rabbits received intravenous infusions of 120 units of thrombin over a period of one hour; blood samples were obtained by cardiac puncture immediately and at 30 and 60 minutes after the termination of the infusion and were examined for proteolytic activity. All rabbits receiving endotoxin or thrombin were sacrificed immediately after the last blood sample had been obtained, and sections of the liver, spleen, kidneys, and lungs were taken for histologic studies.

No spontaneous proteolytic or fibrinolytic activity could be demonstrated in blood samples obtained during the first four hours after a single intravenous injection of endotoxin, while normal blood samples of three rabbits before

endotoxin administration did reveal some fibrinolytic activity, as shown by the complete lysis of euglobulin clots and partial lysis of whole blood clots after 20 hours of incubation. Furthermore, by the fibrin plate assay, less fibrinolysis occurred with blood samples from endotoxin-treated animals then with those from untreated controls. In addition, no proteolytic activity could be found in the blood samples obtained at a time after infusions of thrombin when circulating fibrin would presumably have been present in considerable quantity. When the test animals from both groups were sacrificed after their last bleeding, no significant fibrin deposition was evident on microscopic examination of the visceral organs.

In order to determine whether fibrinolysis might be occurring at the local tissue level, although not demonstrable by *in vitro* techniques, the following experiment was performed.

24 rabbits were given either a single intravenous injection of 0.4 mg of endotoxin or an intravenous infusion of 300 units of thrombin over a 2 hour period. Groups of 3 animals each were sacrificed at 1, 2, 4, and 8 hours after the start of each procedure. Histologic sections of the liver, spleen, lungs, and kidneys were examined for the amount of fibrin deposited in relation to the time of sacrifice.

It was expected that if fibrin were first deposited and then subsequently removed by local enzymatic activity, microscopic examination might reveal increased fibrin accumulation during the early hours with diminution at the later time intervals. However, the microscopic findings disclosed a general paucity of fibrin deposits with no temporal relationship evident. This study did not, of course, rule out the possibility that lysis of circulating fibrin might have been occurring before or simultaneously with its deposition.

None of these experiments provided any evidence for the existence of an active fibrinolytic mechanism in rabbits injected with endotoxin or thrombin. Von Kaulla (22), Westphal (28), and others using *in vitro* methods have also reported failure to demonstrate increased fibrinolytic activity in the plasma of rabbits receiving a single intravenous injection of endotoxin. The weight of available evidence, therefore, argued against the fibrinolytic system functioning as an active determinant in the pathogenesis of the generalized Shwartzman reaction or as a homeostatic mechanism in the removal of circulating fibrin, and suggested that EACA had promoted fibrin deposition by some mechanism other than inhibition of fibrinolysis.

The Role of the Reticuloendothelial System in the Pathogenesis of the Generalized Shwartzman Reaction.—Since a fibrinolytic mechanism was apparently not involved in the reaction to injections of endotoxin or thrombin, it was necessary to postulate some other means for the efficient removal of fibrin formed in the circulating blood. The reticuloendothelial system was next considered, as it is known to function efficiently in the removal of injected particulate matter and colloidal materials from the circulation. Since fibrin and fibrin intermediates

are polymerized macromolecular aggregates, they might be subject to phagocytic removal by the reticuloendothelial cells of the liver and spleen. An intimation of the existence of such a process was provided by Benacerraf and Sebestyen (29) who found that the plasma of rabbits treated with endotoxin contained phagocytizable substances which on intravenous injection competitively inhibited the clearance of carbon in mice. The nature of these substances was not determined, but modified fibrinogen was considered to be a possibility.

It would follow from this concept that any interference with the phagocytic activity of the reticuloendothelial system during sustained intravascular clotting might lead to fibrin accumulation and ultimately to renal cortical necrosis. Good and Thomas (30) may in fact have demonstrated this effect, in showing that prior administration of reticuloendothelial blockading agents such as thorotrast or trypan blue rendered rabbits susceptible to the elicitation of renal cortical necrosis by a single injection of endotoxin, although they interpreted this effect of colloidal materials as an interference with the removal or detoxification of endotoxin by the reticuloendothelial system. Their conclusion was based on the observation that thorotrast given 6 hours *after* instead of *before* an injection of endotoxin did not result in the production of renal cortical necrosis. However, this particular time interval may have been inappropriate since by this time not only had endotoxin been cleared from the blood stream but intravascular clotting may also have subsided and much of the circulating fibrin would already have been removed. Under these circumstances no valid judgment could be made concerning whether thorotrast was acting primarily by enhancing the direct effect of endotoxin or by inhibiting the removal of circulating fibrin. In an attempt to resolve this problem, it was decided to examine more closely the influence of the timing and order of injections of thorotrast and endotoxin on the elicitation of the generalized Shwartzman reaction.

A. Effect of Thorotrast on the Production of Bilateral Renal Cortical Necrosis:

Four groups of rabbits in this experiment received injections of thorotrast either preceding or following single intravenous injections of endotoxin. In Group *a* the rabbits were pretreated with thorotrast 3 hours before the injection of 0.05 mg of endotoxin. The dose of endotoxin was reduced for this particular group to avoid excessive mortality (30). In Groups *b*, *c* and *d* the order of injections was reversed with thorotrast given at time intervals of 1½, 3, or 7 hours after single injections of 0.2 mg of endotoxin. Rabbits used as controls received either thorotrast or endotoxin alone. Those animals surviving to the 24th hour were sacrificed, and autopsies were performed on all rabbits. A summary of the data is presented in Table V.

Howard, Rowley, and Wardlaw (27) demonstrated that the phase of rapid elimination of P^{32}-labeled endotoxin from the circulation was completed well within the first hour following intravenous injection, and other studies (31–33) have yielded similar findings. Consequently, in the present study the administration of thorotrast 1½ to 3 hours *after* an intravenous injection of endotoxin

could hardly be regarded as enhancing the action of endotoxin by prolonging its presence in the circulation. Nevertheless, many animals so treated showed marked fibrin deposition and renal cortical necrosis, with an incidence as high as that shown by those animals which received thorotrast injection before challenge with endotoxin (Figs. 1 and 2). These observations would seem to indicate that the action of thorotrast was primarily directed at intravascular events occurring *subsequent to* the disappearance of endotoxin from the circulation.

If thorotrast promoted intravascular fibrin deposition primarily by inhibiting the phagocytic removal of circulating fibrin rather than by enhancing any direct effect of endotoxin, it should then be possible to demonstrate the consistent production of renal cortical necrosis by intravenous infusions of thrombin

TABLE V

Effect of Thorotrast on the Production of Bilateral Renal Cortical Necrosis

Experimental procedure	No. of rabbits	No. with bilateral renal cortical necrosis
Endotoxin injection at zero time, thorotrast given:		
(*a*) 3 hrs. before endotoxin	7	5
(*b*) 1½ hrs. after endotoxin	10	8
(*c*) 3 hrs. after endotoxin	5	2
(*d*) 7 hrs. after endotoxin	4	0
Thorotrast alone	4	0
Endotoxin alone	8	0

in the presence of reticuloendothelial blockade. The following experiment was devised to test this hypothesis.

B. Effect of Reticuloendothelial Blockade on-the-Production of Renal Cortical Necrosis by Thrombin Infusions:

In this experiment, one group of rabbits received intravenous thrombin infusions started 4 hours after single intravenous injections of thorotrast; in controls physiologic saline solution was substituted for thrombin. In another group, thrombin infusions were begun either 8 or 18 hours after intravenous injections of 0.4 mg of endotoxin. These time intervals were chosen since Benacerraf and Sebestyen (29) had found a progressive depression of reticuloendothelial phagocytic activity during the first 12 hours after an injection of endotoxin with gradual recovery after 24 hours. Some animals received only endotoxin and served as controls. In the next group, rabbits received infusions of a preparation containing thrombin dissolved in a 1 per cent heat denatured albumin suspension; approximately 1.0 gm of denatured albumin was administered to each animal. Controls received only denatured albumin or thrombin. All infusions were given over a period of 3 hours. The concentration of thrombin was 2.0 to 2.5 units/ml, and a total of 250 to 300 units was given to each rabbit. The results are presented in Table VI.

In this experiment reticuloendothelial blockade was produced by three

colloidal agents differing in composition and biological action: thorotrast and endotoxin presumably were toxic for reticuloendothelial cells while denatured albumin (34) caused transient inhibition by competing with other macromolecular substances for phagocytic uptake by the reticuloendothelial system. Regardless of the nature of the blockade, it is apparent from Table VI that in situations where reticuloendothelial function was depressed, intravenous thrombin infusions regularly produced extensive fibrin deposition and renal cortical necrosis. The relevancy of blockade might be questioned in the case of

TABLE VI

Effect of Reticuloendothelial Blockade on the Production of Bilateral Renal Cortical Necrosis by Thrombin

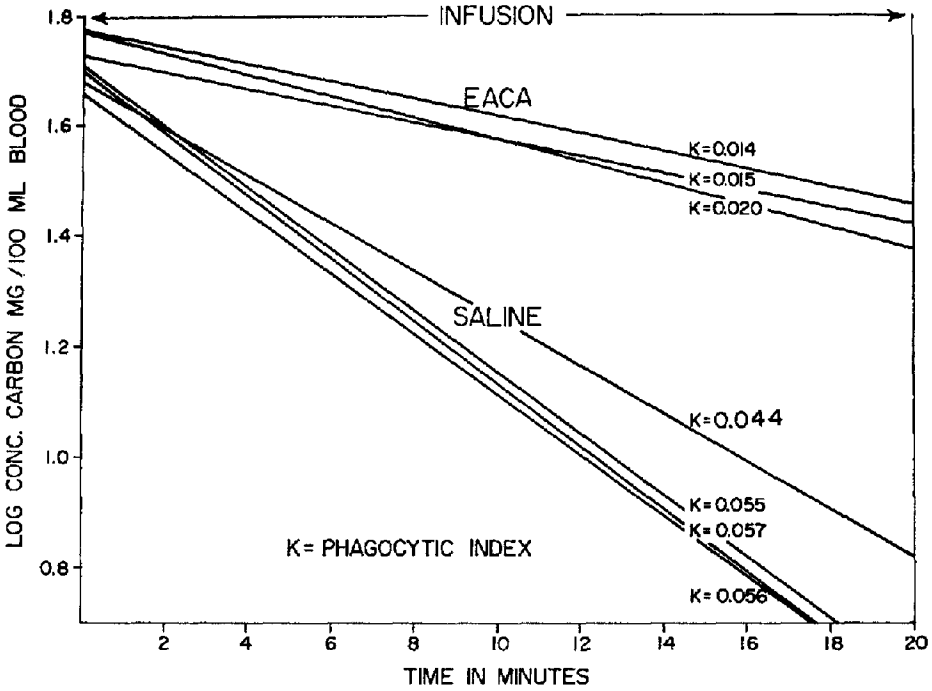
Pretreatment	Intravascular coagulation initiated by	Status of R. E. S. function	No. of rabbits	No. with bilateral renal cortical necrosis
Thorotrast	Thrombin infusion 4 hrs. later	Depressed	19	12
Infusion of denatured albumin	Simultaneous thrombin infusion	Depressed	8	6
Endotoxin	Thrombin infusion 8 hrs. later	Depressed	6	4
Endotoxin	Thrombin infusion 18 hrs. later	Partially recovered	5	0
None	Thrombin infusion	Normal	17	2
None	Endotoxin injection	Normal	8	0
Thorotrast	————	Depressed	8	0
Infusion of denatured albumin	————	Depressed	6	0

Thrombin infusions consisted of 250 to 300 units given intravenously over a 3 hour period. Thorotrast was given in a dose of 3.0 ml/kilograms of body weight. Administration of thrombin at a time when reticuloendothelial function was depressed, either by a prior injection of thorotrast or endotoxin or by simultaneous infusion of denatured albumin, resulted in a high incidence of renal cortical necrosis.

thorotrast and endotoxin, which have other diverse and poorly understood biological activities; however, denatured albumin, possessing little or no pharmacologic activity, in all likelihood produced its effect by competing with circulating fibrin polymers for phagocytic clearance by the reticuloendothelial system. Studies are now in progress aimed at the direct demonstration of fibrin within reticuloendothelial cells of the liver and spleen by immunofluorescence and other techniques.

C. Effect of EACA on the Phagocytic Function of the Reticuloendothelial System: In an earlier section of this paper it was shown that EACA, probably through a mechanism other than inhibition of fibrinolysis, promoted the production of renal cortical necrosis by endotoxin or thrombin. Since reticuloendothelial

blockading agents were found also to promote fibrin deposition during intravascular coagulation, the action of EACA in the production of renal cortical necrosis was reappraised in these terms.

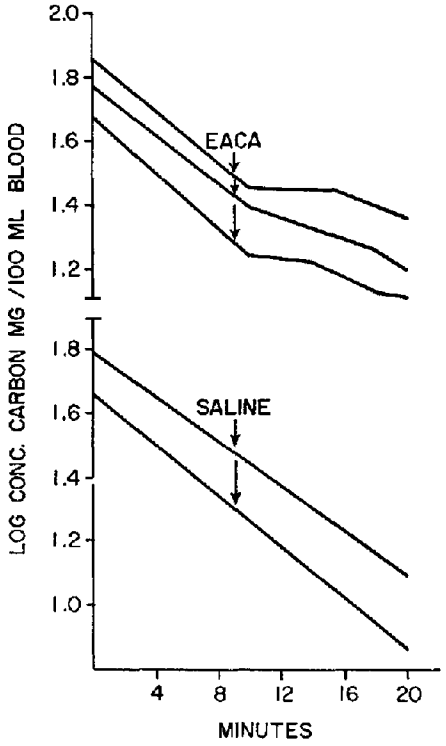


TEXT-FIG. 1. The inhibitory effect of a continuous infusion of EACA on the phagocytic function of the reticuloendothelial system is shown by the significant reduction in carbon clearance rates (K) of EACA-treated rabbits as compared to controls. The upper three curves represent the clearance rates of individual rabbits given intravenous infusions of EACA (40 mg/ml) at a rate of 1 ml/min. during the 20 minute period of the clearance study. The lower four curves represent control animals which received intravenous infusions of physiologic saline solution. The dose of carbon injected at zero time was 4 mg/100 gm of body weight.

The clearance of carbon was measured over a period of 20 minutes with blood samples taken consecutively at two minute intervals from the marginal ear vein. In the first experiment (Text-fig. 1) the clearance of carbon was measured in rabbits during a continuous intravenous infusion of EACA (40 mg/ml) given at a rate of 1 ml/minute. The EACA infusion was started one minute prior to the injection of carbon and was maintained throughout the 20 minutes of the study. In rabbits used as controls, physiologic saline solution was substituted for EACA. In the second experiment (Text-fig. 2) a single intravenous injection of either EACA (1.0 gm/4 ml) or physiologic saline solution was given rapidly within 20 seconds during the 8½ to the 9th minute of carbon clearance.

A significant retardation of the carbon clearance rate was found in rabbits receiving a continuous infusion of EACA (Text.-fig. 1); the average phagocytic

index was reduced to 30 per cent of the value obtained for normal animals. When a single injection of EACA was given in the course of carbon clearance, there occurred within 2 minutes a marked interference with the phagocytosis of carbon particles as indicated by the sudden flattening of the normal clearance curve established prior to EACA administration (Text-fig. 2). In other experi-



TEXT-FIG. 2. The inhibitory effect of a single intravenous injection of EACA on the phagocytic function of the reticuloendothelial system in rabbits is shown by the rapid and marked alteration in the slope of the individual clearance curves when 1.0 gm of EACA was injected during the clearance of carbon. In controls, the injection of 4.0 ml of physiologic saline produced no such changes.

ments, the *in vivo* effect of EACA was found to be transitory since one gram of this material given as a single intravenous injection 15 minutes before the injection of carbon did not influence the rate of clearance. Whether EACA interferes with reticuloendothelial function on a metabolic or a physico-chemical basis requires further clarification.

The Occurrence of Heparin-Precipitable Fibrinogen in the Plasma of Rabbits Infused with Thrombin.—Thomas, Smith, and von Korff (35) found in the blood of endotoxin-treated rabbits an altered form of fibrinogen which precipitated in the cold on the addition of heparin and redissolved on warming the

plasma. This heparin-precipitable fibrinogen (HPF) appeared in the circulation one hour after an intravenous injection of endotoxin, reached maximal quantity at 2 to 4 hours, and was much diminished after 6 hours. It was postulated that HPF represented a stage of polymerization intermediate in the conversion of fibrinogen to fibrin. This concept received support from the work of Shainoff and Page (36) who isolated a cold-precipitable fibrinogen fraction ("cryoprofibrin") from the plasma of endotoxin-treated rabbits, and their analysis of the fibrinopeptide content indicated that this material may represent a product of the action of endogenous thrombin on circulating fibrinogen.

In the present investigation heparinized plasma samples obtained from rabbits during and after intravenous thrombin infusions were also found to contain HPF. The time of appearance of HPF and its persistence in the circulation were studied in four rabbits, each receiving 50 units of thrombin intravenously over a 20 minute period. Heparinized plasma samples (containing 1.0 mg heparin/ml of whole blood) were obtained by cardiac puncture immediately and at hourly time intervals after the termination of the thrombin infusion.

It was found that HPF was present in large amounts in plasma samples obtained during the first hour following thrombin administration. In blood specimens drawn after this time the quantity of HPF diminished progressively, and heparinized plasma samples obtained at the end of the 4th hour showed only minimal precipitation in the cold.

The prompt appearance of HPF following thrombin infusion in contrast to the delayed onset after endotoxin injection may well explain the finding of recognizable fibrin deposits as early as 30 minutes after the start of thrombin administration as opposed to the consistent failure to detect fibrin deposits during the first two hours after the challenging injection of endotoxin. These observations serve to rule out bacterial contamination of the thrombin preparations used here as the causative factor in the production of the renal cortical necrosis observed. Although the presence of HPF appears to be indicative of a transitory thrombin-fibrinogen interaction in the blood stream, its precise nature as well as its relationship to the intravascular fibrin deposits is not fully understood. Further studies bearing on this problem are in progress.

DISCUSSION

It is generally agreed that the essential feature of the tissue damage of the generalized Shwartzman reaction is the occlusion of capillaries by material resembling fibrin. In view of this and other lines of evidence implicating the coagulation mechanism (3–9), it seems likely that, if the Shwartzman reaction had been easily reproducible by intravenous administration of thrombin or thromboplastin, there would have been little reluctance to ascribe a central role to intravascular coagulation in its pathogenesis. In the present study it has been found that while normal animals usually show minimal pathological alterations after infusions of thrombin, rabbits in which reticuloendothelial blockade is combined with infusions of thrombin do indeed manifest all of the

classical features of the generalized Shwartzman phenomenon including typical bilateral renal cortical necrosis. This has permitted the construction of a new and simple hypothesis to explain the pathogenesis of this unique form of tissue damage. According to this hypothesis, the reticuloendothelial system of normal animals functions efficiently in the removal of any circulating fibrin polymers formed in the blood stream. In the generalized Shwartzman reaction, the first intravenous dose of endotoxin initiates intravascular conversion of fibrinogen to fibrin, but this is then quickly cleared from the circulation by the reticuloendothelial system. Subsequently, at a time when the activity of the reticuloendothelial system is much depressed, a second injection of endotoxin again activates intravascular coagulation, but now the fibrin aggregates persist and accumulate in the circulation and are progressively deposited in the terminal vascular bed. This hypothesis seems to be in accord with most of the observations currently available concerning the mechanism of the generalized Shwartzman reaction.

The difficulty in producing bilateral renal cortical necrosis in most species other than the rabbit and the rare occurrence of this pathologic entity in human diseases may possibly be due to the presence in these other species of dual protective mechanisms, both fibrinolysis and reticuloendothelial phagocytosis of fibrin operating to prevent this form of tissue damage. The rabbit may be in the unfortunate position of having only one effectively functioning mechanism, so that any interference with its activity during intravascular clotting leads to disseminated fibrin deposition and the ultimate development of the generalized Shwartzman reaction. Finally, these studies suggest that investigation of the activation of intravascular coagulation, fibrinolysis, and reticuloendothelial function may contribute to the understanding of those human diseases characterized by intravascular deposits of "fibrinoid" or fibrin.

SUMMARY

Intravenous injections of endotoxin or infusions of thrombin in the rabbit initiate intravascular coagulation but do not usually result in massive deposition of fibrin. It has been proposed that the reticuloendothelial system may function efficiently in the removal of circulating fibrin; its blockade permits reproduction of all of the features of the generalized Shwartzman reaction by infusions of thrombin. In the rabbit the reticuloendothelial system may constitute the major protective mechanism against the vasculo-occlusive lesions of the generalized Shwartzman reaction, which appears to be the direct consequence of intravascular fibrin formation and deposition.

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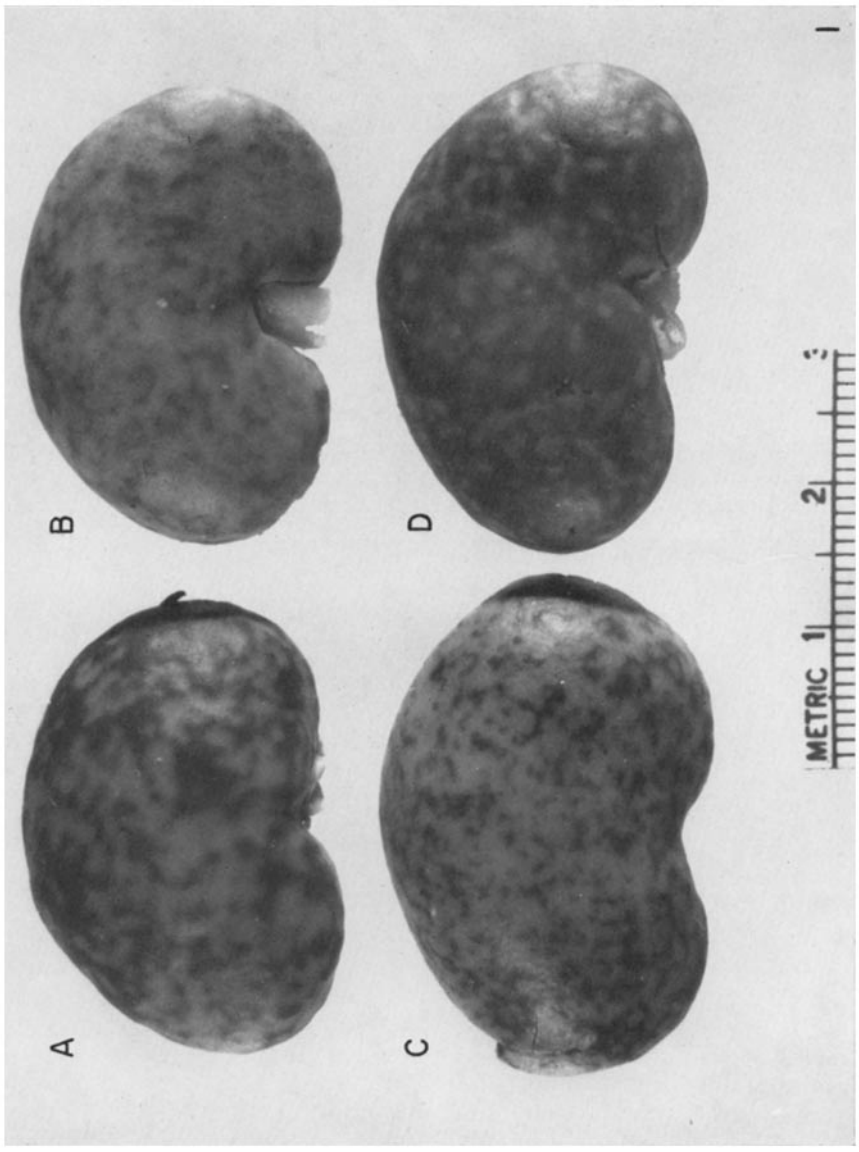
EXPLANATION OF PLATES

PLATE 117

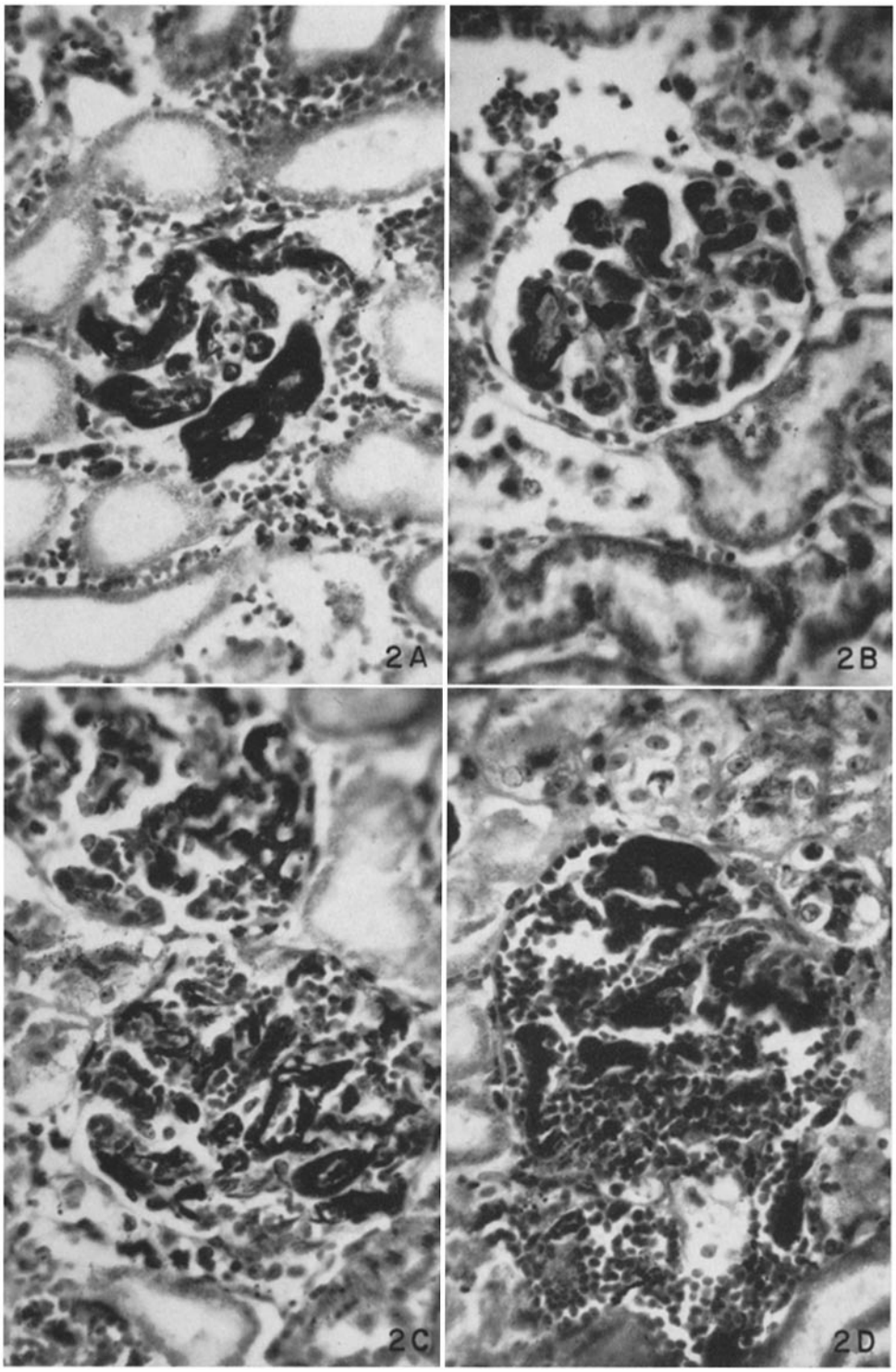
FIG. 1. Gross appearance, after fixation in 10 per cent formalin, of kidneys from rabbits with bilateral renal cortical necrosis sacrificed at the 24th hour of the experiment. Dark foci of hemorrhage and light areas of necrosis cover the entire subcapsular surface of each kidney. *A*, Renal lesion produced by intravenous infusion of 300 units of thrombin given over a duration of two hours (Table I). *B*, Lesion produced by the classical procedure of two intravenous injections of 0.4 mg of *E. coli* endotoxin spaced 16 hours apart. *C*, Lesion produced by 0.4 mg. of *E. coli* endotoxin injected intravenously followed three hours later by an intravenous infusion of 1.5 gm. of EACA (Table III). *D*, Lesion produced by intravenous injection of 0.4 mg. of endotoxin followed three hours later by 4.0 ml of thorotrast given intravenously (Table V). Magnification, × 2.



(Lee: Phagocytic clearance of fibrin)

PLATE 118

FIG. 2. Histologic sections of kidneys corresponding to those shown in Fig. 1 stained with phosphotungstic acid-hematoxylin. The microscopic features were similar in each case and consisted of occlusion of glomerular capillaries by dense amorphous masses of fibrin with necrosis of convoluted tubules. Magnification, × 400.



(Lee: Phagocytic clearance of fibrin)