Bleeding Syndrome in Thrombin-Injected Rats Deficient in Fibrinogen and Antihemophilic Factor. (23496)

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Hemostatic mechanisms may be studied conveniently in rats by microscopic observation of the platelet plugs formed after incising mesenteric arteries or veins(1). In rats made hypofibrinogenemic by the intraperitoneal injection of thrombin(2), platelet plugs either failed to form or were ineffective in arresting bleeding(3). Since fibrin is not histologically demonstrable in the plugs formed in the mesenteric blood vessels of normal rats(3), it seemed unlikely that the low level of fibrinogen was responsible for the defective hemostasis. We, therefore, undertook a more complete study of the clotting factors in thrombin-injected rats to determine whether other deficiencies were present which could account for the abnormal bleeding.

Methods. Male albino rats weighing between 75 and 130 g were used. Twenty-five hundred units of thrombin† (140 mg in 0.37 ml) were injected intraperitoneally into 17 unanesthetized rats. Nine of these were studied one hour after injection and 8, 2 hours after injection. Three control rats received an intraperitoneal injection of 140 mg of bovine albumin and the remaining 17 controls were not injected. After intramuscular administration of nembutal (4 mg/100 g), an exposed mesenteric vein was cut with sharp scissors, and observed for the formation of a platelet plug as previously described(1). Blood was then drawn from the abdominal aorta with a syringe and needle. Prothrombin time was measured by a modification of Quick's method(4). Prothrombin, factor V and factor VII concentrations were determined by modifications of Owren's methods (4). Prothrombin consumption was estimated by measuring the residual prothrombin in serum from blood oxalated after one hour at 37°C(4). Antihemophilic factor (AHF) and plasma thromboplastin component (PTC) were estimated from the amount of prothrombin remaining one hour after recalcifying a mixture of citrated hemophilic or PTC-deficient plasma and a 1:10 dilution of rat plasma(4). Fibrinogen was determined by Quick's modification of the Cullen and Van Slyke method, adapted for the use of 0.5 ml of plasma. Fibrinolytic activity at 37°C was estimated from the lysis time of clotted whole blood and of a mixture of bovine fibrinogen and thrombin with the rat's plasma euglobulin fraction(5). Platelet counts were performed by the direct method(6). Capillary fragility was measured on abdominal skin by Dalldorf's negative pressure method(7).

Results. Platelet plugs formed and bleeding ceased completely between 28 and 140 seconds after incising a mesenteric vein in 8 control rats. The plugs adhered to the vessel and could not easily be removed or fragmented with a forceps. If bleeding was renewed by such manipulation, hemostasis was reestablished more rapidly than after the initial incision.

In all of 11 thrombin-injected rats, bleeding continued for from 5 to 8 minutes before the vein was clamped to avoid exsanguination. Platelet plugs formed in only 3 of these animals (2 rats in the 1-hour group and 1 in the 2-hour group). They were not effective in arresting bleeding and could be readily dislodged or fragmented. Vasoconstriction, observed at the ends of the severed vein in all rats, did not appear to reduce blood flow appreciably, an observation which is in agreement with Hugues' results(8).

Gross autopsy findings on 16 animals which received thrombin included nasal bleeding in 2 and bloody urine in 6. Thymic lymph nodes were hemorrhagic in 8 animals, mesenteric nodes in 2. Two rats had ecchymoses in the diaphragm. No evidence of hemorrhage was

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† Parke-Davis Bovine Topical Thrombin, lot No. 015043-C.

TABLE I. Changes in Clotting Factors One and 2 Hours after Intraperitoneal Injection of 2500 Units of Thrombin.

	Clot formed, No. rats	Lysis time, min.	Fibrinogen, mg %	Prothr. time, sec.	Prothr. conc., sec.	Factor V, sec.	Factor VII, sec.	Serum prothr., sec.	Platelets, $10^6/mm^3$	AHF, sec.
Controls	4 (4)	>1440 (4)	170–238 (4)	13.6 ± 3.4 (16)	21.3 ± 2.7 (16)	21.2 ± 3.9 (16)	24.9 ± 3.8 (16)	68.7 ± 23.3 (9)	1.07 ± .72 (9)	71.0 ± 30.2‡ (7)
1 hr	3 (6)	>1440 (3)	65–211 (6)	20.2–22.6 (3)	25.2 ± 3.1* (8)	29.1 ± 6.2* (8)	34.8 ± 4.5* (8)	45.0 ± 15.7† (9)	.71 ± .16* (9)	21.9 ± 5.3* (5)
2 "	2 (8)	40, 70 (2)	30 (1)	38, ∝ (2)	27.1 ± 4.6* (7)	24.8 ± 6.5 (7)	32.1 ± 4.6* (7)	30.6 ± 7.1† (3)	.43 ± .09* (6)	32.1 ± 8.4* (8)

Avg ± stand. dev. No. of animals given in parentheses.

* Compared with control value, $P < 0.01$. † Compared with control value, $P > 0.02$, < 0.05. ‡ The large stand. dev. is the result of one value of 29 sec. All other values were over 60 sec.

observed in the control animals.

No petechiae formed at a negative pressure of 30 cm Hg in 5 rats tested 2 hours after thrombin injection. However, local subcutaneous hemorrhages were observed in 3 of the injected rats, but in none of the controls.

Determinations of clotting factors are shown in Table I. Because of the small volume of blood available, it was not possible to do all determinations on every animal. Whole blood clotted in 5 thrombin-treated animals and the clot lysed rapidly in 2 of them. Lack of clot formation in 9 rats could be attributed in at least one instance to hypofibrinogenemia; in other instances no clot was noted even when fibrinogen concentration was as high as 85 mg%. This apparent failure of clot formation may have resulted from rapid lysis of small clots. It could not be ascribed to the presence of a potent circulating anticoagulant since clots were produced promptly after addition of purified human fibrinogen to the otherwise incoagulable blood from 5 of these rats. Fibrinolytic activity of the euglobulin fraction of plasma varied from 3 to over 24 hours in both control and test animals, and showed no correlation with whole clot lysis time.

No clots formed in prothrombin time determinations on 8 animals whose whole blood failed to clot. Among the rats whose whole blood clotted, the long prothrombin times of the 3 rats in the 1-hour group can be explained by the decreased concentration of prothrombin and Factors V and VII. The still longer prothrombin times of the 2 rats which showed whole clot lysis were not the result of unusually low concentrations of these factors, and may indicate that fibrinogenolysis had occurred during the brief interval between measurement of whole blood clotting time and prothrombin time.

Residual prothrombin in serum was significantly higher than normal after injection of thrombin. Platelet count decreased, but severe thrombocytopenia did not occur. Antihemophilic factor (AHF) was very much diminished. One hour after thrombin injection, a 1:10 dilution of rat plasma had no greater effect on the prothrombin consumption of he-

mophilic plasma than did saline so that AHF concentration was approximately zero. Some AHF was present in the plasma of rats 2 hours after thrombin injection, but the concentration was still significantly below normal. On the other hand, concentration of plasma thromboplastin component (PTC) was not measurably reduced by thrombin administration.

Discussion. Hypofibrinogenemia which occurs after intravenous injection of thrombin is believed to result from intravascular deposition of fibrin(9,10). Defibrination presumably also occurs in rats injected intraperitoneally with thrombin and probably accounts for the absence of clot formation in many of the animals. No anticoagulant was detected in the 5 rats tested. The occurrence of whole clot lysis in 2 animals suggests that fibrinolytic activity may have been partially responsible for lack of clot formation and reduction in fibrinogen concentration in some rats. Whether fibrinolytic activity resulted from activation of the rat's own plasminogen or of the bovine plasminogen present as a contaminant in the injected thrombin(11) was not determined. The fibrinolytic activity of the euglobulin fraction did not correlate well with the presence of whole clot lysis.

Tissue thromboplastin used during preparation of the thrombin is still present in the final product. Its injection could account for the fall in prothrombin(12,13), factor V(13), and perhaps factor VII. Factor V is activated by thrombin(14), and in the rat, active factor V, or serum accelerator globulin, is rapidly destroyed(15). This may contribute to the decrease in factor V. No greater depression of clotting factors was observed in the rats with whole clot lysis than in those whose clots did not lyse. Consequently, although prothrombin, factor V and factor VII, as well as AHF, can be destroyed by fibrinolysin(16) this enzyme did not seem to be important in diminishing their concentration in these experiments.

High serum concentration of prothrombin was found after thrombin injection. The magnitude of the defect in prothrombin consumption is even greater than is indicated by the data on serum prothrombin alone, since in the thrombin-injected rats, amount of prothrombin present in the plasma was low. Vandenbroucke and Verwilghen observed a decrease in prothrombin consumption after intravenous injection of thrombin in dogs, and attributed it to a defect in thromboplastin formation (17). From our data, it seems evident that deficiency of AHF is responsible for the poor prothrombin consumption. The low level of AHF is not surprising, since others have shown that AHF is inactivated by thrombin, both *in vitro* and *in vivo*(18,19).

The number of abnormalities in the clotting mechanism makes it difficult to determine the cause of the failure in platelet plug formation in thrombin-injected animals. However, the decreases in prothrombin, factor V and factor VII do not seem sufficiently great to prevent platelet plug formation(3). The absence of demonstrable fibrin in normal mesenteric plugs(3) makes it unlikely that the hemostatic defect is the result of the low concentration of fibrinogen or of the occasionally observed fibrinolytic activity. Since the tissue thromboplastin liberated when an exposed mesenteric vein is cut must be small in amount and rapidly washed away, it is possible that the formation of platelet plugs under these circumstances depends upon the formation of blood thromboplastin, for which AHF is required(20).

Summary. One and 2 hours after intraperitoneal injection of 2500 units of thrombin into rats, bleeding from severed mesenteric blood vessels was not arrested and platelet plugs rarely formed. Other manifestations of hemorrhagic tendency were present. Capillary resistance was not diminished. Fibrinogen concentration was low; whole clot fibrinolysis was occasionally observed; prothrombin, factor V, factor VII and platelets were somewhat reduced; PTC concentration was normal; and AHF concentration approached zero. It is suggested that the defect in hemostasis is predominantly the result of AHF deficiency.

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Pathogenesis of Arteriolar Necrosis of Malignant Hypertension.* (23497)

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Fibrinoid degeneration and necrosis of the walls of small arteries and arterioles, typical of the malignant phase of hypertension, have been observed in various tissues and organs of dogs with experimental renal hypertension which is accompanied by impairment of renal excretory function(1,2). The necrotizing arteriolar lesions occur in the rat also, when impairment of renal excretory function accompanies experimental renal hypertension(3). Various views concerning the pathogenesis of the necrotizing lesion, based mainly upon studies carried out on the rat, have been published, and these have been the subject of recent reviews(3,4). One concept of the pathogenesis of arteriolar necrosis which has won wide acceptance is that it is due solely to elevated blood pressure(5-9). Byrom and Dodson(7), have even asserted that sudden, intermittent increases of intra-arterial pressure, induced for only a short period, in normal rats, by a series of rapidly repeated, sudden, forcible, intracarotid injections of warm Ringer's solution, resulted in the production, in a few days, of what they interpreted as arterial and arteriolar necrosis, in the kidneys. It did not occur in kidneys the vascular bed of which had been protected from the sudden rise in intravascular pressure by occlusion of the main renal artery just prior to and during the injections. They expressed the view that, although the necrotizing arteriolar lesions are the result of elevated blood pressure alone, yet, once produced, they might lead to a vicious circle whereby the damaged kidney could perpetuate the existing hypertension. Although these results of Byrom and Dodson (7) have not been confirmed(4), nevertheless this concept has been invoked to explain the pathogenesis of malignant hypertension. It seemed of importance, therefore, to repeat their experiments and, in order to make the test more rigid, it was decided to attempt to produce even greater sudden increases of arterial pressure than were attained by them. The maximum rise reported by Byrom and

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