

## The Hemostatic Defect of Uremia

### II. Investigation of Dogs with Experimentally Produced Acute Urinary Retention

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**T**HERE IS both clinical and experimental evidence to indicate that uremia is sometimes complicated by bleeding. In a previous publication, three patients with acute renal insufficiency were studied from a hemostatic point of view.<sup>1</sup> In addition to clinical evidence of a bleeding tendency, all three were found to have an abnormally long clotting time of whole blood in silicone-treated glassware and a prolonged bleeding time. Evidence of a hemorrhagic tendency has been noted at autopsy in dogs after bilateral nephrectomy.<sup>2</sup> Perirenal sheath hemorrhages were seen in dogs after ureteral ligation and phthalylsulfathiazole therapy, but these animals did not have laboratory evidence of a hemostatic defect.<sup>3</sup> In the present study, acute fatal urinary retention was produced by ligation of the ureters of otherwise normal dogs. Presence of a hemostatic defect was demonstrated by detailed studies as the uremia progressed.

#### METHODS

Fifteen healthy adult mongrel dogs of both sexes weighing 10 to 15 Kg. were used. Under sodium pentobarbital anesthesia, bilateral flank incisions were made to expose the ureters. A polyethylene catheter was inserted into each ureter near the renal pelvis and sutured into place. The catheters were brought out through the skin and the incision closed. Urine was allowed to flow through the catheters for the first 24 hours to allow the dog to recover from the effect of surgery and anesthesia. The catheters were then tied externally and no food or water was allowed from that point on.

As the uremia progressed, the hemostatic mechanism was studied by standard procedures described previously.<sup>1</sup> Control values were established by testing each dog on at least 3 occasions prior to surgery. Blood was obtained from the jugular vein using the two-syringe technic. Blood from the first syringe was mixed immediately with 0.1 M sodium oxalate in a ratio of 1 part oxalate solution to 9 parts whole blood. Clotting time and prothrombin consumption studies were done on blood collected in the second (silicone-coated) syringe. Platelet counts were done on blood flowing freely from the venipuncture needle. In experiments where whole blood from two dogs was mixed, silicone-coated glassware was used throughout. Blood was obtained from the two animals simultaneously and placed in separate tubes in an ice bath. Aliquots were transferred to individual tubes with silicone coated pipets. After all mixtures were completed, the tubes were placed in a 37 C. water bath and clotting times determined. Less than 5 minutes elapsed from the time of venipuncture to transfer to the 37 C. bath.

The effect of plasma and serum on the clotting time of whole blood from the uremic dogs was determined. Serial dilutions of plasma or serum were made with 0.85 per cent sodium chloride. Sodium citrate or oxalate was added to the diluting fluid in amounts equal to that of the plasma being tested so that anticoagulant concentration would remain constant.

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Plasma and serum to be tested were obtained from normal dogs and from human patients known to be deficient in AHG, PTC<sup>4</sup> and PTA.<sup>5</sup> In some experiments, plasma from normal dogs was treated with barium sulfate (50 mg./ml.) or tricalcium phosphate (4 mg./ml.). In a series of silicone-treated tubes, 0.1 ml. of the various dilutions was placed. Blood was obtained from a uremic dog and 1.0 ml. was placed in each tube. Clotting time of each mixture was then determined.

Prothrombin consumption during clotting of whole blood was measured by determining the serum prothrombin time 60 minutes after clotting.<sup>1</sup> Serum prothrombin levels were determined at more frequent intervals in two dogs. Prior to the addition of anticoagulant, blood was allowed to stand in a 37 C. water bath for 15, 30 and 60 minutes after venipuncture. Prothrombin activity was then determined by both the one and two-stage methods.<sup>6</sup> Capillary fragility was estimated by means of a negative pressure petechiometer (Rexall Drug Co., Los Angeles, Calif.). The amount of negative pressure necessary to produce 1 to 2 petechiae on the shaved skin of the abdomen was determined. Bleeding times were determined by the method of Adelson.<sup>7</sup> Antihemophilic activity was determined by the method of Langdell, Wagner and Brinkhous.<sup>8</sup> Plasma of a known human hemophilic was used as the substrate.

### RESULTS

The dogs became acutely ill and died 4 to 7 days after ligation of the ureteral catheters. Clinical evidence of a bleeding tendency was not seen in any of the dogs. Six of the dogs were autopsied, and showed varying degrees of terminal pneumonia, pulmonary congestion and hydronephrosis. Evidence of a hemorrhagic tendency was minimal, but consisted of a few scattered subserosal petechial hemorrhages in all six animals; strongly guaiac positive material in the gastrointestinal tract of 2 animals; and moderate sized retroperitoneal hemorrhages in the renal areas of one dog.

The dogs had a similar course and the two experiments summarized in table 1 are typical of the entire group. There was no detectable change in the hemostatic mechanism for the first few days after ligation of the catheters. *Blood urea nitrogen* rose progressively and by the fifth to sixth day ranged from 150 to 250 mg. per 100 ml. of plasma. *Clotting time of whole blood in siliconized tubes* became prolonged in all of the dogs. By the fourth to fifth day more than 65 minutes were required for coagulation to occur. The clotting time became more prolonged in the animals surviving the longest, so that seven had maximal clotting times greater than 2 hours and 2 had clotting times greater than 5 hours. *Clotting time of whole blood in plain glass tubes* became prolonged only moderately. During the uremic phase, clotting times ranged from 8 to 25 minutes. In the three dogs surviving the longest, clotting times greater than 15 minutes were obtained.

*Plasma Prothrombin* remained constant throughout in seven of ten dogs studied (e.g., dog #5 table 1). In the other three, moderate hypoprothrombinemia occurred (e.g., dog #1 table 1). Expressed in terms of their respective control, prothrombin in these dogs ranged from 50 to 75 per cent as measured by both the 1 and 2-stage methods. *Prothrombin consumption* was followed as the uremia progressed in 11 dogs. During these studies, the platelet count remained in the normal range. In all 11 dogs, the serum prothrombin time, determined 60 minutes after clotting, decreased as the uremia progressed. In only the three animals surviving the longest were serum prothrombin times less than 11 seconds. Prothrombin in the serum was determined at more frequent intervals in 2 dogs. When blood from a normal dog is tested in this manner, only a

TABLE 1.—*Effect of Experimental Urinary Retention on Hemostasis in Two Dogs*

Determination	Normal Range*	Dog #1 Days After Ligation			Dog #5 Days After Ligation		
		4	5	6	3	4	5
		1. Blood urea nitrogen (mg. %) . . .	12-21	93	142	145	103
2. Clotting time—Silicone (min.) . . .	15-45	45	110	200	40	80	135
3. Clotting time—Glass (min.) . . . .	3-13	11	13	15	11	12	10
4. Prothrombin time (% of normal). . .	100-85 (5-6.5 sec.)	100	78	66	100	100	100
5. Serum prothrombin time (sec.) . . .	14-55	53	28	13.5	45	38	12
6. Fibrinogen (mg.%) . . . . .	180-280	380	525	455	800	540	420
7. Bleeding time (min.) . . . . .	1-2	1	1½	1	1	1	1½
8. Platelets (1000/mm <sup>3</sup> ) . . . . .	200-480	319	320	270	300	400	410

\* 15 dogs studied on 3 separate occasions each prior to surgery.

trace of prothrombin is present in the serum of blood allowed to clot for 15 minutes in either plain glass or siliconized tubes. The two dogs were studied at a time when their whole blood clotting time in siliconized glassware was greater than 70 minutes and their serum prothrombin time 60 minutes after clotting was in the normal range. Eighty to 100 per cent of the plasma prothrombin activity was present in the serum of blood allowed to clot for 15 minutes in plain glass and 45 minutes in siliconized glassware.

*Labile factor* and *stable factor* levels remained constant throughout the experiments. *Fibrinogen* was consistently higher after surgery and remained high after ligation of the ureteral catheters. During the uremic phase fibrinogen levels ranged from 300 to 800 mg. per 100 ml. of plasma. *Fibrinolysis* activity did not increase. With both normal and uremic animals, clots incubated with their sera for 24 hours at 37 C. contained as much fibrin as clots tested immediately after clotting. *Antithrombin* activity of plasma remained constant throughout the period of observation. One ml. of plasma from both normal and uremic dogs inactivated 80-100 units of thrombin in 2 hours. Thrombin (Topical Thrombin, Parke, Davis) was used in a concentration of 200-250 units/ml. in the incubating mixture. *Antihemophilic activity* was determined on two uremic dogs. Plasma obtained when the whole blood clotting time in siliconized tubes was greater than 80 minutes had the same corrective effect on the plasma from a known human hemophilic as did plasma from a normal dog. *Capillary fragility* was no different in the animals after ligation of the ureteral catheters than in the normal dogs. *Bleeding time* was no longer than 1½-2 minutes in either the normal or uremic dogs.

*Platelets* were in the normal range throughout the period of observation in 9 animals studied. In order to evaluate platelet function, whole blood from uremic animals was mixed with whole blood from dogs made severely thrombocytopenic by hypothermia.<sup>8</sup> The experiments, summarized in table 2, indicate that there was mutual correction of the prolonged clotting time of whole blood in silicone-treated tubes associated with the two conditions. However, blood from the uremic dog was not quite as effective as was blood from the normal dog in correcting the prolonged clotting time of the thrombocytopenic blood.

The possibility that a circulating anticoagulant is responsible for the pro-

TABLE 2.—*Silicone Clotting Time of Mixtures of Thrombocytopenic Blood with: (A) Uremic Dog Blood and (B) Normal Dog Blood*

Uremic blood (ml.) Thrombocytopenic* blood (ml.)	0 2.0	0.2 1.8	0.5 1.5	1.0 1.0	1.5 0.5	1.8 0.2	2.0 0
A							
Clotting time (min.)							
Experiment 1	180	180	55	40	30	40	120
Experiment 2	160	—	55	45	40	30	95
B							
Clotting time (min.)							
Experiment 3	120	60	50	40	35	30	30
Experiment 4	120	85	55	25	30	25	25

\* platelet count 9,000–17,000 per cu.mm. caused by severe hypothermia.<sup>8</sup>

TABLE 3.—*Silicone Clotting Time of Mixtures of Uremic and Normal Dog Blood*

Uremic (ml.) Normal (ml.)	0 2.0	0.8 1.2	1.2 0.8	1.4 0.6	1.8 0.2	2.0 0
Clotting time (min.)						
Experiment 1	25	20	15	15	40	70
Experiment 2	25	30	35	30	40	120
Experiment 3	15	25	35	145	145	145
Experiment 4	30	35	60	65	90	90
Experiment 5	30	15	40	95	300	300
Average	25	25	45	70	125	145

longed clotting time in siliconized tubes was tested by using mixtures of normal and uremic dog blood. The results of 5 experiments indicate that blood from uremic dogs does not inhibit clotting of normal blood (table 3). Small amounts of normal blood completely corrected the prolonged clotting time in 2 dogs (experiments 1 and 2, table 3). Larger amounts of normal blood were required for complete correction of the prolonged clotting time in the other 3 dogs (experiments 3, 4 and 5, table 3). In other studies, clotting times in silicone tubes were determined on mixtures of whole blood from the uremic dogs and various dilutions of plasma or serum in saline. Although low concentrations of normal and various abnormal plasmas and sera completely corrected the prolonged clotting time, equivalent volumes of saline had the same corrective effect. Tocantins<sup>9</sup> has shown such an effect by dilution on the clotting time in silicone of both normal and abnormal blood.

Urea has been shown to dissolve fibrin clots,<sup>10</sup> and thus might inhibit coagulation. Serial two-fold dilutions of a 6 per cent solution of urea were made and 0.1 ml. aliquots were placed in silicone-treated tubes. To each tube, 1.0 ml. of whole blood from a normal dog was added and the clotting time determined. All tubes contained solid clots in 20 minutes although blood to which no urea was added required 35 minutes to clot. In another experiment, blood urea nitrogen was elevated by placing 20 Gm. of urea in solution into a dog's stomach immediately after ligation of the ureteral catheters. The BUN rose from the normal

level of 15 mg. per 100 ml. to 206 mg. during the first 24 hours. The clotting time of this blood in siliconized tubes was 40 minutes. Twenty-four hours later when the BUN was 166 mg., the clotting time was 45 minutes.

#### DISCUSSION

Acute urinary retention as produced in these experiments results in a hemostatic defect which becomes more pronounced as the uremia progresses. Prolongation of the clotting time of whole blood in siliconized glassware appears to be the earliest sign of the developing hemorrhagic tendency. An abnormally long clotting time of whole blood is generally accepted as evidence of a coagulation defect. For example, the major manifestation of a recently described coagulation factor is that in its absence the clotting time of blood is long.<sup>11</sup> On this basis, it could be said that the hemostatic defect associated with acute uremia is the lack of a previously undescribed clotting factor, since minute amounts of plasmas deficient in known coagulation factors correct the prolonged clotting time. On the other hand, the results of the whole blood mixing experiments suggest an excess of a coagulation inhibitor.

Bleeding time prolongation and clinical manifestations of a hemostatic defect occurred approximately 10 days after the onset of anuria in the human.<sup>1</sup> In contrast, the dogs did not develop bleeding time prolongation or clinical evidence of a hemostatic defect. The data suggest that the duration of urinary retention is a major factor in the pathogenesis of the hemostatic defect. Since the dogs died four to seven days after ligation of the ureteral catheters, anuria may not have been present a sufficient length of time to influence these tests. Additional studies with less acute experiments are needed to clarify this point.

The prolonged clotting time of whole blood associated with hypothermia is corrected less efficiently by blood from a uremic dog than by blood from a normal dog. This suggests that platelet function may be impaired in acute uremia since it has been shown that thrombocytopenia is the basis of the hemostatic defect in hypothermia.<sup>8</sup> However, if diminished platelet function were the cause of the prolonged clotting time in acute uremia, one would expect little or no correction by thrombocytopenic blood.

Direct inhibition of coagulation by urea does not appear to be a factor in acute uremia but azotemia may have an indirect inhibitory effect. It seems possible that the accumulation of "waste products" could cause minor alterations in the configuration of one or more of the coagulation proteins. Such a modification could make the substance relatively inert in the weak activator system of clotting whole blood, but in the presence of a strong activator such as brain thromboplastin, normal response could occur. There is general agreement that a qualitative defect in platelets can occur, but additional studies are necessary in order to evaluate the concept of a qualitative defect of coagulation proteins.

#### SUMMARY

Acute urinary retention was produced in 15 adult mongrel dogs by ligation of the ureters. Studies of the hemostatic mechanism were made as the uremia progressed. All animals developed a coagulation defect characterized by ab-

normally long clotting time of whole blood in siliconized glassware. Other indices of coagulation efficiency were found to be normal or only slightly impaired.

It is not clear whether the basic defect is a lack of an essential clotting factor or an excess of an inhibitor. Urea does not seem to be the cause of the defect. The data suggest that the duration of urinary retention is as important as the degree of azotemia.

#### SUMMARIO IN INTERLINGUA

Acute retention urinari esseva producite in 15 adulte canes hybrida per ligation del ureteres. Studios del mecanismo hemostatic esseva executate durante que le uremia progrededa. Omne le animales disveloppava un defecto coagulatori characterisate per un anormalmente longe tempore de coagulation de sanguine integre in vitros a revestimento de silicium. Altere indices del efficacia coagulatori se monstrava normal o non plus que levemente prejudicate.

Il non es clar si le defecto fundamental es un carentia de un indispensabile factor coagulatori o un excesso de un inhibitor. Il pare que urea non es le causa del defecto. Le datos colligite suggere que le duration del retention urinari es tanto importante como le grado de azotemia.

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