Acquired Factor IX Deficiency in the Nephrotic Syndrome

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In a patient with the nephrotic syndrome prolonged whole blood clotting and defective intrinsic thromboplastin generation resulted from an acquired deficiency of factor IX. His urine and that from eight additional patients with massive proteinuria, in contrast to normal urine, contained a procoagulant with factor IX activity. The extraction of this substance as a barium sulfate adsorbate from urine is described, and its ability to correct the abnormality in thromboplastin generation of patients with congenital factor IX deficiency and elicit rabbit antisera that inactivate human serum factor IX is demonstrated. These findings support excessive urinary loss of factor IX as a potential cause for acquired factor IX deficiency in certain patients with massive proteinuria.

In 1967 Handley and Lawrence (1) described acquired factor IX deficiency in four children with the nephrotic syndrome. Corticoid therapy in each child brought about a reduction of urinary protein content and a simultaneous increase in serum factor IX activity. Therefore, the authors suggested but could not confirm that the clotting disorder resulted from excessive urinary loss of factor IX.

The present report documents urinary loss of factor IX as a potential cause for factor IX deficiency. The presentation of an additional patient with the nephrotic syndrome in whom prolonged whole blood clotting resulted from a reduction in factor IX activity illustrates a uniquely acquired defect in blood coagulation.

Case Report

A 17-year-old negro boy entered the hospital in July 1969 because of anemia and edema. He described episodic abdominal pain and recurrent jaundice since early childhood. Four years previously hemoglobin electrophoresis had disclosed homozygous-S disease. At the present admission he had conjunctival pallor, scleral icterus, and anasarca but no ecchymoses or petechiae.

His hematocrit value was 18% and total leukocyte count, 20,000/mm^3. Differential cell count showed 70 segmented neutrophils, 1 eosinophil, 25 lymphocytes, and 4 monocytes. A direct platelet count was 440,000/mm^3 and reticulocyte count, 19%. Peripheral blood film showed numerous sickle cells, occasional target cells, and 10 normoblasts/100 leukocytes. Blood urea nitrogen was 11 mg/100 ml; serum creatinine, 0.9 mg/100 ml; serum cholesterol, 587 mg/100 ml; and total serum lipids, 1,236 mg/100 ml. Total serum protein concentration was 4.2 g/100 ml with an albumin fraction of 1.0 g/100 ml. Plasma fibrinogen level was 380 mg/100 ml. Protein content of the urine varied from 15 to 25 g/24 hr. Urinary sediment contained oval fat bodies and large waxy casts. Intravenous pyelogram showed kidneys of normal size and shape.

Routine blood clotting studies obtained before contemplated percutaneous renal biopsy showed a whole blood clotting time (Lee-White) of 20 min and bleeding time (Ivy) of 2 min. Prothrombin time was 13.4 sec with a control value of 12.4 sec. A partial thromboplastin time was 72 sec (normal value, less than 45 sec). These results prompted additional medical history that disclosed that at age 14 the patient underwent multiple extractions of teeth without excessive or prolonged hemorrhage. Neither he nor any family member recalled an unusual bleeding episode.

To specifically identify the blood clotting defect in this patient we performed a thromboplastin generation test. A defect in the serum component caused his 6-min thromboplastin generation time to be prolonged (Table 1). Admixture of normal serum with his serum completely corrected the abnormal thromboplastin generation. By contrast, admixture of his serum with that of a person with congenital factor IX deficiency caused no change in the prolonged thromboplastin generation time. These findings excluded the presence of a circulating anticoagulant and confirmed that the patient's coagulation disorder resulted from factor IX deficiency. They also persuaded his attending physicians to defer renal biopsy.

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Table 1. Thromboplastin Generation in a Patient with the Nephrotic Syndrome

Generation Time	Clotting Times		
	2 min	4 min	6 min
		sec	
Control	30	13	11
Patient plasma / Patient serum	90	60	50
Patient plasma / Normal serum	25	14	11
Normal plasma / Patient serum	80	70	63
Patient plasma / Patient serum: normal serum (1:1)	26	18	11
Patient plasma / Patient serum: factor IX-deficient serum (1:1)	75	70	54

Initially the patient sustained a moderate diuresis from therapy with furosemide. Because of severe and unremitting proteinuria he received prednisone, 60 mg daily, beginning in September 1969. After 1 month of this therapy there was no reduction in his 24-hr urinary protein content, but a partial thromboplastin time measured 44 sec, and his thromboplastin generation test also became normal. A specific assay done at this time showed a serum factor IX level 70% of normal.

His physicians then discontinued corticoid therapy because of its failure to lessen the proteinuria. Two weeks later the partial thromboplastin time again was prolonged at 57 sec. One month after withdrawal of corticoid drugs a partial thromboplastin time was 61 sec and prothrombin time, 12.9 sec. Specific assay now quantitated his serum factor IX level at 20% of normal.

Materials and Methods

We performed coagulation testing on venous blood collected in plastic syringes and clotted in siliconized glassware or anticoagulated in a 1:10 volume of 0.1 M sodium oxalate.

The procedures of one-stage and two-stage prothrombin times followed the methods of Quick (2), using commercial thromboplastin (Hyland Laboratories, Costa Mesa, Calif.). Russell's viper venom (Sigma Chemical Co., St. Louis, Mo.) substituted for brain thromboplastin in the Stypven time. Our technique for the activated partial thromboplastin time was that of Proctor and Rapaport (3), using a commercial phospholipid (Platelin® plus activator, Warner Chilcott Laboratories, Morris Plains, N. J.).

For the thromboplastin generation test we used the basic procedure of Biggs and Douglas (4) with substitution of a prepared platelet substrate (5) (Platelin); barium sulfate served as the plasma adsorbant. The specific factor IX assay consisted of a two-stage method, following the scheme of Biggs and MacFarlane (6).

Special Studies

To confirm excessive urinary loss of factor IX as the cause for acquired factor IX deficiency in our patient we attempted to isolate a unique procoagulant with factor IX activity from his urine. This required not only concentration of potential factor IX activity from a large volume of urine but avoidance of a naturally occurring urinary procoagulant, P-substance (7-11). Barium sulfate, which removes factors II, VII, IX, and X from a calcium-free solution by adsorption, does not specifically or firmly adsorb P-substance (11-12). Thus, looking for factor IX activity, we studied the procoagulant activity of a barium sulfate adsorbate of urine from our patient, 10 normal subjects, and 15 additional patients with proteinuria.

We collected all urine specimens in iced plastic containers. Their processing began with addition of 0.15 M sodium oxalate, 10 ml/100 ml urine, followed by adjustment of the urinary pH to 7.5 with 10 N sodium hydroxide or 10 N hydrochloric acid, or both. After removing both calcium as the oxalate salt and cellular debris by centrifugation at 2,500 rpm for 15 min, we added barium sulfate, 200 mg/100 ml urine. Constant agitation at room temperature maintained the insoluble barium sulfate in suspension for 15 min. We then deposited the barium sulfate by centrifugation at 2,500 rpm for 10 min and washed it with three 50-ml portions of chilled, 0.85% sodium chloride.

We eluted the barium sulfate adsorbate at room temperature for 20 min with 5 ml of 0.2 M sodium citrate. After removal of citrate by dialysis in 0.1 M ammonium acetate for 18 hr at 10 C, we studied the eluate for procoagulant activity in the thromboplastin generation and one-stage prothrombin time tests. Occasionally, lyophyllization of the dialyzed eluate and brief storage at −20 C preceded coagulation testing.

Additionally, we immunized one rabbit with dialyzed barium sulfate adsorbate from our patient's urine and another rabbit with similar material from urine of a second individual with massive proteinuria (Patient 1, Table 2). Immunization consisted of four subcutaneous injections with an emulsion of adsorbate in Freund's adjuvant given at 2-day intervals. Each rabbit received a total of about 50 mg protein as the adsorbate. We harvested control sera before immunization and immune sera 3 weeks after the initial injection.

Results

The patient presented and three others with massive proteinuria manifested abnormal blood coagulation consistent with acquired factor IX deficiency during the course of their illnesses. Barium sulfate adsorbates of urine samples from 9 of 10 patients with a 24-hr urinary protein content above 10 g contained procoagulant activity that corrected the abnormality in thromboplastin generation of a patient with congenital factor IX deficiency (Table 2). In contrast, we detected no such procoagulant activity in urine from 6 patients with lesser amounts of proteinuria and from 10 normal subjects.

Figure 1 demonstrates the correction afforded by addition of urinary barium sulfate adsorbates from two nephrotic patients (Patients 5 and 10, Table 2) to abnormal thromboplastin generation tests of

Table 2. Correlation of Clinical and Laboratory Findings with Urinary Factor IX Activity in Patients with Proteinuria

Patient	Age/Sex	Clinical Diagnosis	Urinary Protein	Serum Albumin	Serum Creatinine	Urinary Factor IX Activity	Prothrombin Time Patient/Control	Partial Thromboplastin Time
			g/24 hr	*g/100 ml*	*mg/100 ml*		*sec*	
1	56/M	Amyloidosis	20-25	1.0	2.5	Present	13.5/12.6	42.0
2*	17/M	Sickle cell nephropathy	15-25	1.0	0.9	Present	13.4/12.4	72.0
3*	36/F	Idiopathic nephrotic syndrome†	15-20	1.2	2.4	Present	12.0/12.4	200.0‡
4	36/M	Membranous glomerulonephritis†	15-20	1.1	1.8	Present	12.8/12.4	38.0
5	56/F	Idiopathic nephrotic syndrome†	16-19	1.0	1.9	Present	11.9/12.0	36.8
6*	18/F	Proliferative glomerulonephritis†	12-18	1.2	0.7	Present	12.4/12.4	68.3
7*	43/M	Proliferative glomerulonephritis†	10-16	1.2	1.7	Present	12.8/12.0	63.0
8	16/M	Sickle cell nephropathy†	10-15	2.3	1.6	Present	12.2/11.8	39.8
9	56/F	Membranous glomerulonephritis	8-12	1.7	7.5	Absent	12.6/12.0	37.8
10	29/F	Idiopathic nephrotic syndrome†	5-10	1.1	0.7	Present	12.0/11.8	38.6
11	52/F	Proliferative glomerulonephritis†	4-7	2.3	1.0	Absent	12.6/12.2	
12	61/F	Membranous glomerulonephritis†	2-6	3.2	0.7	Absent	12.0/12.0	
13	31/M	Cystinuria	2-3	4.1	1.3	Absent	12.6/12.0	
14	38/M	Membranous glomerulonephritis†	2-3	4.2	8.7	Absent	12.8/12.4	
15	15/F	Postrenal transplant	0.5-1.5	4.0	2.4	Absent	11.9/12.2	38.0
16	24/M	Asymptomatic proteinuria†	0.8-1.0	4.4	1.1	Absent	12.2/12.0	

* Abnormal blood coagulation tests consistent with acquired factor IX deficiency.
† Histological diagnosis established by renal biopsy.
‡ Partial thromboplastin time without kaolin activation, normal value less than 100 sec.

both congenital hemophilia B and the patient presented with acquired factor IX deficiency (Patient 2, Table 2). Although one 24-hr urinary adsorbate exhibits less procoagulant activity than the other, their factor IX activity per microgram of adsorbate protein is similar. In four adsorbates tested (Patients 1, 2, 5, and 10, Table 2), 3 to 7 μg protein added to the thromboplastin generation of a patient with congenital factor IX deficiency resulted in a normal 6-min test. Total factor IX activity contained in two 24-hr urinary adsorbates from the patient presented, before he received corticoids, approximated that found in 250 ml and 300 ml of normal human serum. These adsorbates failed to shorten prolonged one-stage prothrombin and Stypven times of normal aged plasmas or plasmas from patients taking coumarin drugs. Moreover, they could not substitute for platelets or factor VIII in the thromboplastin generation test. Thus, we found no activity of factors V, VII, VIII, or X or platelets in any of these urines that contained substantial factor IX activity.

The instability to heat of this urinary procoagulant and the resultant loss of its factor IX activity (Figure 2) closely resembles that property of human serum factor IX. Plasma factor IX manifests a similar heat instability curve (13).

Figure 3 demonstrates inactivation of the serum reagent in normal thromboplastin generation by rabbit antisera obtained through immunization with urinary adsorbates from two patients exhibiting massive proteinuria. These antisera do not prolong the normal one-stage prothrombin time or affect the ability of aged serum to partially correct the prolonged prothrombin time of plasmas from patients receiving coumarin drugs. Thus, they lack the capacity to inhibit factors VII or X and seem directed primarily against factor IX. Their addition to normal plasma reduces by about 25% the amount of thrombin generated in a two-stage prothrombin assay, implying some slight inactivation of factor II.

The immunoelectrophoretic pattern shown in Figure 4 resulted from interaction of a serum concentrate of factors VII, IX, and X with one of the aforementioned antisera. It appears similar to the immunoelectrophoretic pattern formed by an antiserum to factor IX elicited with plasma antigens (14). Prior mixture of our antiserum with normal human serum containing factor IX prevents forma-

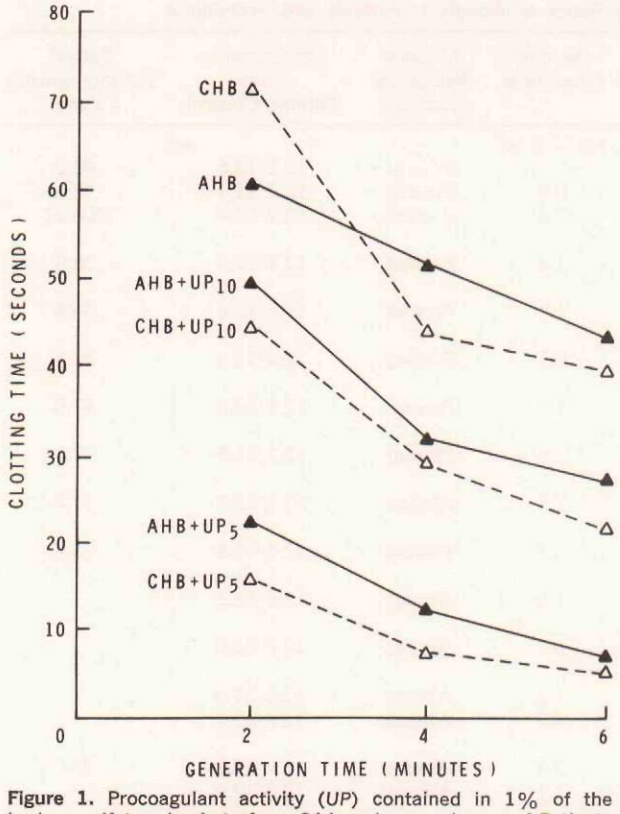

Figure 1. Procoagulant activity (*UP*) contained in 1% of the barium sulfate adsorbate from 24-hr urine specimens of Patients 5 and 10, Table 2, provides complete and partial correction of abnormal thromboplastin generation in congenital hemophilia B (*CHB*) and acquired factor IX deficiency (*AHB*, Patient 2, Table 2).

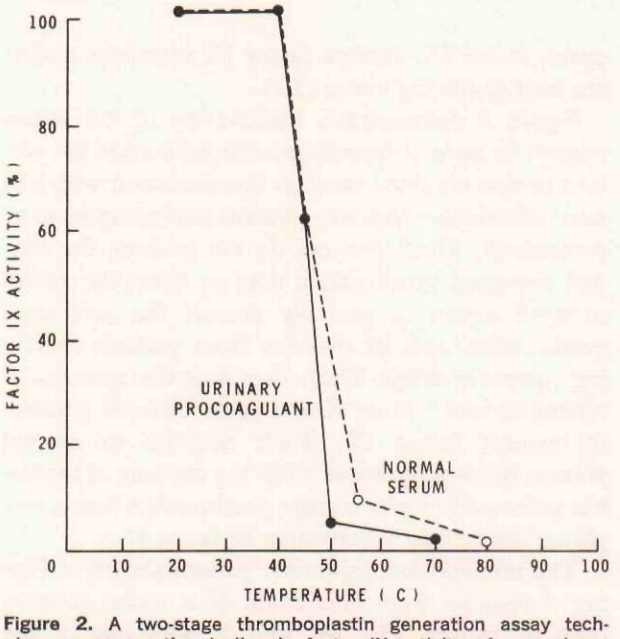

Figure 2. A two-stage thromboplastin generation assay technique measures the decline in factor IX activity of our urinary procoagulant and normal human serum after incubation for 10 min at various temperatures.

tion of the precipitin arc. The arc remains if the human serum mixed with the antiserum first is made free of factor IX by adsorption with barium sulfate.

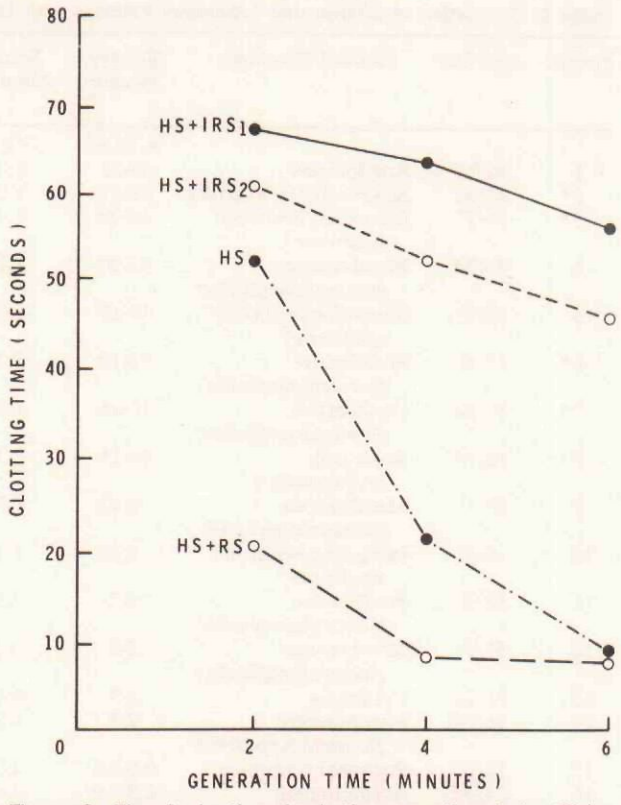

Figure 3. The 6-min thromboplastin generation test utilizing normal human serum (*HS*), a 1:1 admixture of this serum with control rabbit serum (*RS*), and similar admixture with immune rabbit sera (*IRS*) elicited by urinary adsorbates of Patients 1 and 2, Table 2. Repeated adsorption with barium sulfate removed factor IX from all rabbit sera.

That the precipitin band is single also suggests lack of cross-reaction between the anti-IX moiety and factors VII and X.

Discussion

The results of this investigation confirm factor IX deficiency as an acquired blood coagulation defect in certain patients with the nephrotic syndrome and provide insight into the mechanism responsible for depletion of this clotting factor.

We have shown that a substance that corrects the abnormality in thromboplastin generation of patients with both congenital and acquired factor IX deficiency appears as a unique component in urine from certain patients with massive proteinuria. Heat instability similar to that of human factor IX first distinguished the activity of this substance from that provided by a naturally occurring urinary procoagulant, P-substance (7-11). P-substance, which may be derived from the renal parenchyma, also substitutes for factor IX in coagulation testing but, unlike human factor IX, resists significant loss of this activity even at temperatures approaching 100 C (9). P-substance frequently is absent entirely from urine

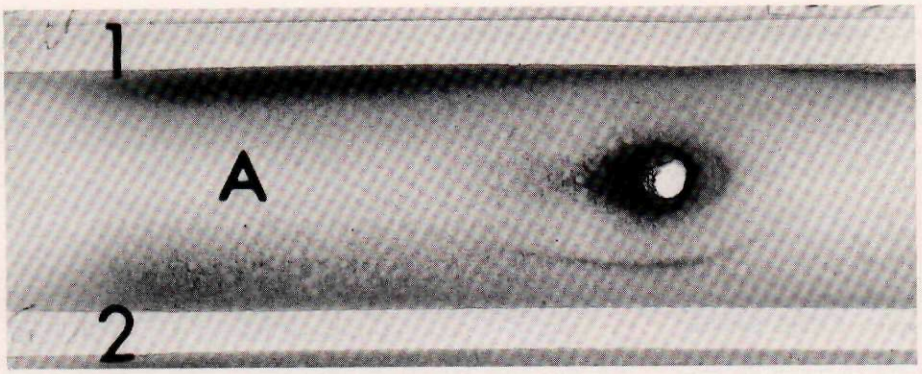

Figure 4. The central antigen well (A) contains a barium sulfate adsorbate of human serum concentrating factors VII, IX, and X about 25 times their normal serum levels. Trough 1 contains immune rabbit serum (IRS_1) previously mixed with normal human serum. Trough 2 contains IRS_1 previously mixed with barium sulfate adsorbed human serum. Anode is to the left.

of nephrotic patients; when present its concentration is inversely related to the urinary content of protein (10). Finally, our urinary procoagulant elicits rabbit antisera that inhibit human serum factor IX and form an immunoelectrophoretic pattern similar to that described for plasma factor IX. These observations offer confirmatory evidence that the urinary procoagulant from our patients is indeed factor IX.

The appearance of factor IX deficiency in patients with the nephrotic syndrome seems directly related to the severity of the disorder as estimated by total urinary protein content and serum albumin concentration. We did not observe abnormal blood coagulation tests in such patients unless their 24-hr urinary excretion of protein exceeded 15 g on at least one occasion, typically in association with a serum albumin level of 1.2 g/100 ml or less (Table 2). Furthermore, factor IX activity was absent from urines containing less than 7 g protein per 24-hr specimen but consistently appeared in urine from 9 of 10 patients whose urinary protein content amounted to or exceeded 10 g/24 hr.

Why factor IX appears to be the only coagulation factor depleted by urinary loss in patients with massive proteinuria might be related to a relatively small molecular size of this factor. Previous estimates of the molecular weight of factor IX vary from 100,000 to 200,000 (15-16), although autoprothrombin II, a purified derivative of the prothrombin complex that may be factor IX, has a molecular weight of 49,900 (17). Urinary loss of factor II may also occur in these patients but is not necessarily implied by the slight reduction in prothrombin activity after addition of the rabbit antisera to the two-stage prothrombin assay. Even recent, sophisticated attempts to separate the antigens of factor II from those of factor IX have only been partially successful (18). Indeed, similar antigenic groups may be present on the two factors (19).

All abnormal blood clotting tests in our four patients with acquired factor IX deficiency reverted to normal after corticoid therapy. In three of our four patients as well as in each of those previously reported (1) substantial reduction in urinary protein content occurred with return of abnormal blood coagulation tests to normal. In contrast, the patient presented here in detail sustained no remission in proteinuria after treatment with corticoids. Nevertheless, at the time his coagulation studies improved, demonstration of factor IX activity in his urine became inconstant.

Importantly, none of our patients or those described by Handley and Lawrence (1) manifested unusual bleeding despite their grossly abnormal blood clotting studies. This probably is related to the degree of factor IX depletion. In four of five patients studied the circulating factor IX activity remained above 10% of normal. At these levels of activity even posttraumatic bleeding seldom occurs in patients with congenital factor IX deficiency (6).

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