

## SYNDROME OF ACQUIRED FACTOR X DEFICIENCY AND SYSTEMIC AMYLOIDOSIS

## In Vivo Studies of the Metabolic Fate of Factor X

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**Abstract** To determine the metabolic fate of factor X in primary amyloidosis associated with factor X deficiency, we examined the pathways of its catabolism in a man with this syndrome. Intravenous infusion of human or bovine  $^{131}\text{I}$ -labeled factor X established a triphasic plasma clearance pattern for factor X. About 85 per cent of the factor X disappeared, with a disappearance half-time of less than 30 seconds. A second and third phase showed a  $T_{1/2}$  of 90 minutes and nine hours respectively.  $^{131}\text{I}$ -labeled factor X in

plasma did not appear to be rapidly modified or degraded. Relatively minor quantities of  $^{131}\text{I}$  were cleared into the urine. We observed a diffuse distribution of radioactivity over the body surface, with a concentration in the hepatic and splenic regions. These studies demonstrate that factor X deficiency associated with systemic amyloidosis is due to binding of factor X to body tissue, probably within the circulatory system. (N Engl J Med 297:81-85, 1977)

AN acquired form of factor X deficiency has been described in association with systemic amyloidosis.<sup>1-9</sup> These patients do not have circulating inhibitors of blood coagulation and do not respond to either vitamin K or infusion of plasma. Although in some patients treatment is not effective, others have been reported to respond temporarily to infusion of factor X concentrates of intermediate purity.<sup>6,8,9</sup> The disease appears to be characterized by enhanced destruction rather than by decreased synthesis of factor X. These patients have usually succumbed rapidly to the bleeding diathesis or to amyloid infiltration of vital organs. To evaluate a therapeutic strategy employing bovine factor X for replacement therapy in a disorder refractory to the administration of human factor X, we undertook studies with human and bovine factor X to determine the metabolic fate of infused factor X. Our data demonstrate that both human and bovine factor X are neither rapidly degraded nor rapidly excreted into the urine; rather, factor X is cleared from the circulating plasma and probably becomes immobilized within the vasculature.

## CASE REPORT

A 49-year-old man had a myocardial infarction in 1972, bradycardia and first-degree heart block documented in 1974 and a bleeding duodenal ulcer in 1975. He came to the New England Medical Center Hospital in March, 1976, with bilateral calf pain and a recent history of mucous-membrane bleeding, weight loss and muscle weakness. There was no similar problem among members of his family. Evaluation elsewhere had identified an isolated factor X deficiency. The patient appeared to be robust and vigorous. Relevant findings included an irregular pulse at a rate of 56 per minute,

modest hepatosplenomegaly and flaccid quadriceps and calf muscles. No sensory or motor deficits were observed.

Laboratory data included a hemoglobin of 13.1 g per deciliter, hematocrit of 38 per cent, white-cell count of 11,000, with a normal differential, and platelet count of 615,000. Red-cell morphology was unremarkable. The blood urea nitrogen was 13 and creatinine 0.8 mg per deciliter, and the alkaline phosphatase 6.0 Bodansky units. Urinalysis was normal except for 1.8 g of protein per 24 hours. Coagulation studies showed a prothrombin time of 33.5 (control, 13.3), partial thromboplastin time of 72 (control, 43), thrombin time of 9.0 (control, 8.5) and Stypven time of 22.5 seconds (control, 13.0) and bleeding time (template) of 3.5 minutes. Activities of specific coagulation proteins, as compared to pooled normal plasma with activity defined as 1.0 U per milliliter, were factor X (0.045 U per milliliter), factor XII (0.95), factor XI (0.90), factor IX (1.05), factor VIII (2.00), factor VII (2.25), factor V (1.05) and prothrombin (1.14); antithrombin III was 1.0 U per milliliter, and fibrinogen 1212 mg per deciliter. A circulating anticoagulant was not detected. The plasma levels of factor X in the patient's mother were 0.83, in his father 1.18, in a sister 0.74, in a brother 1.40, in a son 0.91 and in a daughter 0.91 U per milliliter.

Serum protein electrophoresis revealed a small M component identified as IgG kappa by immunoelectrophoresis. Serum gamma-globulin levels were IgG, 3.9, IgA, 0.42, and IgM, 0.32 mg per milliliter. A bone-marrow aspirate was normal except for the presence of 8 per cent morphologically normal plasma cells.

Studies of nerve conduction demonstrated decreased conduction velocities and diminished amplitude of evoked potentials, consistent with a diffuse peripheral neuropathy. An electrocardiogram disclosed first-degree heart block and left-bundle-branch block and suggested the possibility of an old lateral infarction. The PR interval was 0.36 second. Multiple premature ventricular contractions were observed, and a short episode of ventricular tachycardia was documented. X-ray study indicated cardiomegaly. In the face of acquired factor X deficiency and abnormalities of nerve and cardiac conduction, the suspicion of amyloidosis was confirmed by biopsy of the rectal mucosa. Amyloid, which stained with Congo red and displayed green birefringence under polarized light, was observed in the subendothelium of the venuoles, arterioles and some capillaries. A bone-marrow biopsy later in the course also demonstrated amyloid deposition in the sinusoids.

There were intermittent episodes of hypotension and bradycardia secondary to heart block, but implantation of a permanent pacemaker was precluded because of the coagulopathy. Therefore, a systematic approach was taken to understand the pathophysiology of this disorder so as to identify a method of temporarily correcting the coagulopathy to permit operation. The studies described pertain to this effort.

After the diagnosis was established, the patient's condition gradually deteriorated. On melphalan and prednisone, he had continued weight loss, severe sporadic muscle pain involving the quadriceps and gluteal region, and occasional hypotension. He died in December, 1976, with severe anasarca, liver failure, and hyperkalemia.

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## METHODS AND MATERIALS

**Preparation of labeled factor X.** Factor X was prepared from fresh bovine plasma<sup>10,11</sup> and outdated human plasma<sup>12</sup> according to published methods. Factor X was iodinated with Na <sup>131</sup>I (New England Nuclear) with use of chloramine-T.<sup>13</sup> The <sup>131</sup>I-labeled factor X was separated from free <sup>131</sup>I on columns of Sephadex G-25 (Pharmacia). The labeled protein was recovered quantitatively and retained full factor X activity. Samples for infusion were sterilized by passage through a Millipore Swinnex filter (pore size of 0.22  $\mu$ m) and mixed aseptically with a human serum albumin solution. This material was infused within one hour of preparation.

**Plasma and urine clearance studies.** <sup>131</sup>I-labeled human factor X and <sup>131</sup>I-labeled bovine factor X were infused with the informed consent of the patient and after review of the experimental protocol by the Human Experimentation Committee and the Radiation Committee at the New England Medical Center Hospital. After premedication with potassium iodide, we infused <sup>131</sup>I-labeled factor X intravenously through an antecubital vein and removed blood samples into plastic tubes containing citrate from an indwelling venous cannula in the opposite arm.

The plasma clearances of <sup>131</sup>I-labeled bovine factor X and native bovine factor X were compared in separate infusion studies in rabbits. In both experiments, factor X was infused into the right-ear vein, and blood samples were removed into plastic tubes containing citrate from the vein of the opposite ear. In the experiment employing <sup>131</sup>I-labeled factor X, plasma aliquots were assayed for <sup>131</sup>I. In the experiment involving infusion of unlabeled factor X, bovine factor X in the rabbit plasma was assayed with the Laurell rocket-immunoelectrophoresis method<sup>14</sup> employing rabbit anti-bovine factor X hyperimmune serum. Normal rabbit plasma did not form precipitin bands against rabbit anti-bovine factor X immune serum. The bovine factor X concentration in the rabbit plasma after infusion was quantitated by comparison with normal rabbit plasma supplemented with known quantities of bovine factor X.

**Total-body radioactive scanning.** Body scans were performed manually with a Ludlum Measurements model 12 count-rate meter (Sweetwater, Texas). Scans were repeated daily with placement of the crystal over regions of the anterior body surface. <sup>131</sup>I scintigraphy was performed with an Ohio-Nuclear radioisotope camera and an iodine high-energy collimator.

**Radioactive counting.** Samples containing <sup>131</sup>I were assayed using a Baird-Atomic Spectrometer model 530 equipped with a model 810 scintillation detector and corrected for the radioactive decay of <sup>131</sup>I.

We performed disc gel and sodium dodecyl sulfate gel electrophoresis with standard methods.<sup>15,16</sup> Gels were either stained for protein with amido black or anazoline sodium (Coomassie Blue) or sliced to facilitate counting of radioactive <sup>131</sup>I-labeled factor X.

## RESULTS

**Studies with human factor X.** The plasma clearance rates of coagulation proteins have usually been measured by quantitation of enzyme activity in the plasma after infusion of the enzyme into a subject deficient in the enzyme, monitoring of plasma radioactivity levels of the radiolabeled plasma protein infused into a normal or deficient subject, or measurement of the decrease in enzyme activity as a function of time after complete inhibition of protein synthesis, as with the vitamin K antagonists in normal subjects. Each of these methods has its assumptions and ambiguities. For purposes of establishing the fate of factor X to develop a rational approach to therapy for our patient, we initially studied plasma factor X turnover using the first two methods outlined.

**Plasma infusion studies.** We infused 1 liter of normal fresh frozen plasma containing 1000 U of factor X intravenously over 30 minutes after plasmapheresis of 600 ml of plasma. Whole-blood samples were removed in citrate. We determined the prothrombin time, partial thromboplastin time, and factor X level and observed minimal changes in these coagulation assays as a function of time after infusion. A similar experiment was performed on a larger scale with use of plasmapheresis to facilitate infusion of 4000 U of factor X daily. The patient was subjected to plasmapheresis for three consecutive days, each involving the exchange of 4000 ml of patient plasma for normal fresh-frozen plasma with use of a Haemonetic model 30 blood processor (Haemonetics Corporation, Na-

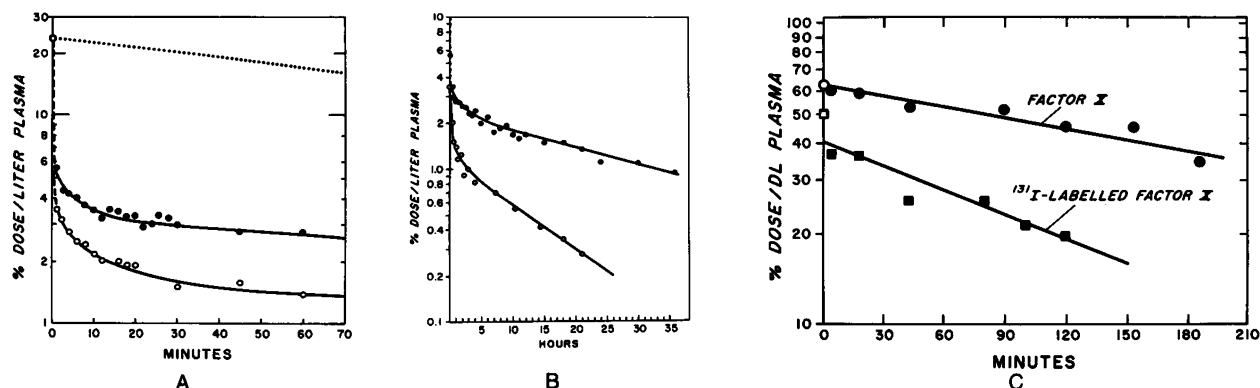


Figure 1. Plasma Levels of <sup>131</sup>I after Infusion of <sup>131</sup>I-Labeled Factor X.

The percentage of the infused <sup>131</sup>I dose appearing in the patient plasma is plotted on a logarithmic scale as a function of time after infusion. The initial point at zero time (0) was calculated on the basis of total plasma volume. ○—○ shows <sup>131</sup>I-labeled human factor X, and ●—● <sup>131</sup>I-labeled bovine factor X. For comparison, the predicted plasma clearance of factor X in a normal subject is shown indicated by the dotted line.<sup>17,18</sup> A shows a detail of the first 70 minutes of the experiments. B a plot of data obtained for 1.5 days after infusion, and C a comparison of the plasma disappearance of labeled and unlabeled bovine factor X infused into rabbits. <sup>131</sup>I-labeled bovine factor X (3.6  $\mu$ g) or unlabeled factor X (5.2 mg) was infused and plasma levels of <sup>131</sup>I-labeled factor X (■—■) and unlabeled factor X (●—●) were determined by assay of <sup>131</sup>I and Laurell rocket immunoelectrophoresis, respectively. The percentage of the infused factor X appearing per deciliter of plasma is plotted on a logarithmic scale as a function of time. The open symbols at time 0 are calculated estimates of the percentage dose per deciliter of plasma based upon the estimated plasma volume.

tick, Massachusetts). After completion of each exchange, the plasma factor X activity was measured and remained unchanged. These experiments indicated that factor X activity disappeared from the plasma so rapidly that measurement of the plasma half-life by this method was precluded.

*Plasma infusion of  $^{131}\text{I}$ -labeled human factor X.* We infused  $^{131}\text{I}$ -labeled human factor X (12  $\mu\text{g}$  containing 1 mCi) and removed samples of patient plasma and assayed them for  $^{131}\text{I}$  activity as a function of time. These results, expressed as the percentage of the infused dose of  $^{131}\text{I}$  per liter of plasma, are shown in Figure 1. The point at time 0, the theoretical maximum of the plasma  $^{131}\text{I}$  level, is calculated from the estimated plasma volume (40 ml per kilogram of body weight) and the total amount of radioactive label infused. The plasma disappearance of  $^{131}\text{I}$ -labeled factor X follows a complex rate process. This disappearance may be interpreted with a triphasic model in which three processes dominate. In the first phase, approximately 85 per cent of the infused factor X disappeared from the plasma, with a disappearance half-time of less than 30 seconds. In the second phase, involving approximately 10 per cent of the infused factor X, factor X was cleared by a process compatible with first-order kinetics with a  $T_{1/2}$  of 90 minutes. A third phase, involving approximately 5 per cent of the infused factor X, consisted of clearance of factor X by a process compatible with first-order kinetics with a  $T_{1/2}$  of 10.5 hours. Approximately 85 per cent of the  $^{131}\text{I}$  in the plasma was precipitable with trichloroacetic acid and 70 per cent of the  $^{131}\text{I}$  in the plasma bound to barium citrate. Gel electrophoresis of the plasma proteins that absorbed to and eluted from barium citrate, monitored with  $^{131}\text{I}$ , migrated with the same electrophoretic mobility as native human factor X or  $^{131}\text{I}$ -labeled human factor X on gel electrophoresis in dodecyl sulfate. These results suggest that the circulating plasma  $^{131}\text{I}$  remains bound to factor X that has not been altered proteolytically.

The urine excreted after plasma infusion of  $^{131}\text{I}$ -labeled factor X was collected and assayed for radioactivity. The patient excreted 12.6, 4.4 and 3.1 per cent of the infused  $^{131}\text{I}$  on the first, second and third days respectively. In contrast to the plasma, 90 per cent of the  $^{131}\text{I}$  excreted into the urine was not precipitable by trichloroacetic acid, suggesting the absence of intact factor X in the urine. These results indicate that urinary losses do not account for the rapid plasma clearance of factor X in this disorder.

Rapid factor X clearance from the plasma in the face of the appearance of relatively minor quantities of  $^{131}\text{I}$  in the urine was interpreted as suggestive of the sequestration of factor X in body tissues. We determined the distribution of factor X by monitoring anatomic regions for  $^{131}\text{I}$ .  $^{131}\text{I}$  radioactivity was quantitated over the anterior body surfaces daily with a portable count-rate meter. Although the  $^{131}\text{I}$  measured included circulating  $^{131}\text{I}$ -labeled factor X, as well as that bound to tissue, the rapid clearance of factor X from

the blood suggested that tissue-bound factor X was the predominant source of radioactive isotope measured. As shown in Figure 2, the manual quantitation of tissue-bound  $^{131}\text{I}$  detected factor X throughout the body surface with some apparent concentration in the right and left upper quadrants of the abdomen. In a study extending over a seven-day period, the tissue-bound radioactive label over the hepatic region had a biologic half-life of about 2.7 days.

*Studies with bovine factor X.* These initial studies indicated that human factor X is cleared from the plasma and is rapidly immobilized. As a first hypothesis, we envisioned that a tissue-bound binding site, perhaps the amyloid matrix, recognized certain structural features of human factor X and bound circulating factor X. Despite a high degree of similarity between factor X and prothrombin, prothrombin was not recognized and bound, as was factor X. This observation led us to examine whether bovine factor X possessed the structural requisites for interacting with the tissue-binding sites.

In vitro studies using patient plasma indicated that the plasma coagulation defect was completely correctable with the addition of purified bovine factor X, as with human factor X. The partial thromboplastin time and prothrombin time of the patient's plasma

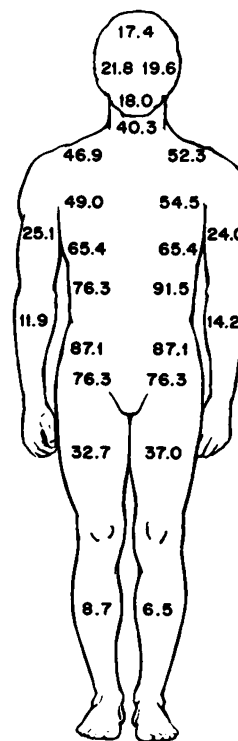


Figure 2.  $^{131}\text{I}$  Activity over the Anterior Body Surface. Twenty-four hours after infusion of 1 mCi of  $^{131}\text{I}$ -labeled human factor X, radioactivity was quantitated manually over the anterior body surface with a portable count-rate meter. The numbers on the figure refer to counts per minute, in thousands, which we obtained by placing the crystal directly onto the skin surface.

were normal with the addition of factor X to a final concentration of 5  $\mu\text{g}$  per milliliter of plasma. These studies indicated that there was no circulating inhibitor of factor X, and suggested that bovine factor X might be useful *in vivo* to treat the coagulopathy. We considered the possibility that a plasma protein in the patient's plasma might bind to factor X without inhibiting factor X activity. This complex might be rapidly cleared *in vivo*. To examine this possibility disc gel electrophoresis was performed on patient and normal plasma preincubated separately with  $^{131}\text{I}$ -labeled bovine factor X; the labeled factor X migrated identically in either plasma. From these studies, we consider it unlikely that any plasma protein in the patient's plasma binds tightly to factor X.

*Plasma infusion of  $^{131}\text{I}$ -labeled bovine factor X.* To test the hypothesis that bovine factor X might not possess the structural requisites for tissue binding that human factor X contains, we performed clearance studies using  $^{131}\text{I}$ -labeled bovine factor X (30  $\mu\text{g}$  containing 1 mCi) infused intravenously. As with human factor X, 80 per cent of the infused bovine factor X was rapidly cleared from plasma, with a disappearance half-time of less than 30 seconds (Fig. 1). A second phase, consistent with first-order kinetics, had a  $T_{1/2}$  of 90 minutes. A third phase, also consistent with first-order kinetics, had a  $T_{1/2}$  about 12 hours. Approximately 97 per cent of the plasma  $^{131}\text{I}$  was precipitable by trichloroacetic acid, and 90 per cent of the plasma  $^{131}\text{I}$  could be absorbed to barium citrate. Comparison by sodium dodecyl sulfate electrophoresis of  $^{131}\text{I}$ -labeled factor X, factor X and a patient plasma fraction prepared by barium citrate absorption and elution followed by ammonium sulfate fractionation demonstrated that the infused  $^{131}\text{I}$ -labeled factor X circulated in an undegraded form.

Because of ethical considerations of the risks of human experimentation with a procoagulant, a parallel experiment involving infusion of  $^{131}\text{I}$ -labeled factor X into a normal human subject was precluded. For these reasons, we performed studies in rabbits to demonstrate that factor X was not altered by labeling with Na  $^{131}\text{I}$  in such a way as to enhance its plasma clearance rate.  $^{131}\text{I}$ -labeled bovine factor X (3.6  $\mu\text{g}$  containing 120  $\mu\text{Ci}$ ) was infused intravenously into a New Zealand white rabbit. Initial measurements of  $^{131}\text{I}$ -factor X in the plasma were 80 per cent of that predicted on the basis of the infused dose and the estimated plasma volume of the animal. In the initial phase, factor X was cleared from the plasma by a process characterized by first-order kinetics with a half-time of about 110 minutes (Fig. 1C). This clearance time corresponds favorably to the  $T_{1/2}$  of factor X in man during the initial phase of plasma clearance.<sup>17,18</sup> To demonstrate directly that  $^{131}\text{I}$ -labeled bovine factor X and unlabeled bovine factor X are cleared at the same rate, thereby emphasizing the validity of estimating factor X clearance rates with use of  $^{131}\text{I}$ -labeled factor X, bovine factor X (5.2 mg) was infused into a rabbit, and the plasma clearance of bovine factor X from rabbit plasma determined. As shown in Figure 1C, the

plasma bovine factor X concentrations, determined by rocket immunoelectrophoresis, were altered as a function of time corresponding to a  $T_{1/2}$  of about 240 minutes. The plasma clearance of  $^{131}\text{I}$ -labeled bovine factor X and unlabeled bovine factor X in rabbits appear similar, considering that the discrepancies may be related to intrinsic difference between the rabbits or the methods employed for detection. However, in comparison to the rapid clearance of  $^{131}\text{I}$  from the patient's plasma (disappearance half-time <30 seconds), these experiments indicate that the clearance of  $^{131}\text{I}$ -labeled factor X from the patient's plasma *in vivo* is not related to structural alterations of factor X caused by iodination.

Further investigation of the fate of  $^{131}\text{I}$  infused into the patient revealed that approximately 25 per cent of  $^{131}\text{I}$  infused as  $^{131}\text{I}$ -labeled bovine factor X was excreted into the urine over the first three days. This radioactivity was not trichloroacetic acid precipitable, suggesting that the  $^{131}\text{I}$  was either free or bound to peptide degradation fragments of factor X.

Radioactivity measurements of  $^{131}\text{I}$  over the anterior body surface were obtained as in experiments with human factor X. The magnitude of the activity, the distribution of the activity and the decay of the activity were similar to those in experiments performed with human factor X. Two days after infusion of  $^{131}\text{I}$ -labeled bovine factor X, we performed imaging studies, using an Ohio-Nuclear Radioisotope Camera. Scans of the thorax and abdomen indicated increased uptake in the thyroid, liver, spleen and bladder superimposed upon generalized soft-tissue activity.

## DISCUSSION

Factor X is a circulating plasma zymogen involved in an intermediate stage of blood coagulation. Bovine factor X has a molecular weight of 56,000, contains two polypeptide chains whose amino acid sequence is known, contains two metal-binding sites and has a three-dimensional structure probably homologous to trypsinogen and chymotrypsinogen.<sup>11,19-24</sup> Human factor X has not been extensively characterized, but from inferences extrapolated from the marked similarities of bovine and human prothrombin, human factor X is likely to show marked resemblance to bovine factor X.<sup>25</sup> The plasma disappearance of human factor X after infusion has two phases. The first phase, perhaps representing equilibration of the intravascular and extravascular compartments, has a  $T_{1/2}$  of about 1.5 to three hours. The second phase, perhaps representing protein degradation, has a  $T_{1/2}$  of about 48 hours.<sup>17,18</sup> An isolated absence of factor X activity in human plasma is usually related to congenital factor X deficiency. However, acquired factor X deficiency can be associated with systemic amyloidosis in a rare syndrome about whose pathophysiology little is known. Previous therapeutic attempts have demonstrated that patients with this disorder are relatively refractory to infusion of human plasma components containing factor X or to treatment with vitamin K. These attempts have suggested that the inability to elevate the plas-

ma level of factor X activity for an appreciable time is related to either rapid plasma degradation of factor X, renal clearance of factor X, or binding of factor X directly to amyloid infiltrates within the tissues. To study the pathophysiology of this syndrome, we performed plasma turnover studies using highly purified human and bovine factor X. Infusion studies with radioactively labeled factor X demonstrated that the bulk of factor X is cleared from the plasma at a rate so rapid that most of the factor X appears to be removed on the first transit through the circulation.

Because of our inability to perform a control infusion experiment in a normal human subject, we relied on indirect studies to support our implicit hypothesis that the plasma clearance of infused  $^{131}\text{I}$ -labeled factor X reflects the plasma clearance of native, unlabeled factor X. The iodination of factor X was not associated with changes in biologic factor X activity or changes in its electrophoretic mobility on polyacrylamide gels; these results are inconsistent with major structural alterations or aggregation in the factor X preparation. Furthermore, the similarity of the initial kinetics of plasma disappearance of factor X, labeled and unlabeled, in the rabbit as compared to patterns established in normal man, is in marked contrast to the results obtained in this patient with amyloidosis. Lastly, the infusion of an aggregated or colloidal preparation of  $^{131}\text{I}$ -labeled factor X would be characterized by uptake exclusively in the reticuloendothelial system with a plasma disappearance half-time of about three to four minutes determined by the quantity of the blood volume that passes through the liver and spleen per minute.

Circulating plasma factor X is removed at a rapid rate and apparently immobilized within the vasculature since the binding sites for factor X must be exposed to the circulating plasma to permit rapid exchange. These binding sites are probably distributed diffusely, with the appearance of concentration in the region of the liver and spleen owing to an increased density of microvasculature in those regions. The chemical nature of the binding sites is unknown, but one might speculate that the highly organized amyloid deposits in the subendothelium and perhaps the endothelium may, through its quaternary structure, have a high affinity and specificity for factor X. The structural requisites for factor X that facilitate its interaction with the binding site are not currently known. Although bovine factor X and prothrombin share many physicochemical properties and have marked structural homology, prothrombin does not appear to interact strongly with these sites. On the other hand, the structural and chemical differences between the human and bovine forms of factor X do not appear to affect the nature of the interaction of factor X with the binding sites. In particular, bovine factor X does not seem to represent a useful component for the rational treatment of this coagulopathy. A detailed study in vitro of the interaction of factor X

with affected tissue or purified amyloid protein should provide understanding of the relation of factor X structure and the amyloid ultrastructure.

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