# RECURRENT THROMBOEMBOLISM: REPORT OF A CASE AND INQUIRY INTO THE NATURE OF THE FAST ANTIPLASMIN

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Errata:

p 1018 - fig. 4 0: rabbit antihuman serum should read:
rabbit antihuman alpha$_2$-macroglobulin serum

and: when precipitant bands of dilutions of the patient's plasma against alpha$_2$-macroglobulin should read:
against rabbit antihuman alpha$_2$-macroglobulin.

# Recurrent thromboembolism: Report of a case and inquiry into the nature of the fast antiplasmin

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Thrombosis is usually the expression of more than one defect. If these defects are no longer present when the thrombosis becomes clinically manifest, it becomes almost impossible to reconstruct its development. Not infrequently, apparently spontaneous, recurrent, or progressive thrombosis occurs without clear-cut abnormalities of the vascular wall, the venous circulation, or the coagulation mechanism, and therefore the cause of the disease remains unknown. In contrast many cases of thromboembolism develop secondary to polycythemia vera, thrombocythemia, or carcinoma of the prostate, lung, or pancreas, thus suggesting a definite etiologic relationship.

Of interest is the association of recurrent thrombosis to increased antiplasmin activity of blood or plasma noted by Naeye[20] and by Nilsson and co-workers.[21, 22] Increased antiplasmin activity has been observed in a number of patients with thromboembolic complications or with frank intravascular coagulation. The nature of this relationship is presently far from clear. Furthermore, the antiplasmin activity responsible for the observed abnormalities has not been precisely defined. This study shows that increase in *fast* antiplasmin appeared to be responsible for development of thromboembolism in a woman with nephrosis. This antiplasmin activity was found to be associated with the alpha$_2$-macroglobulin fraction of plasma or serum.

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## CASE REPORT

Mrs. M. G. (University of Minnesota Hospitals No. 105-01-05), a 29-year-old octipara, was admitted April 3, 1966, in her sixth month of pregnancy with pedal edema and marked proteinuria. Previous pregnancies have been normal. There was no history of urinary tract infections. Blood pressure on admission was 130/80; pulse rate was 88 and regular. Eye grounds were normal. Except for 2+ pedal edema and 3+ proteinuria, examination was essentially negative.

Laboratory findings included normal blood urea nitrogen (BUN) and creatinine values and a negative lupus erythematosus (L.E.) clot test; Addis count was $1.7 \times 10^6$ red blood cells and $5 \times 10^6$ leukocytes per 12 hours with no casts. Serum electrophoresis revealed abnormal protein patterns (Table I.) Serum cholesterol was 386 mg. percent.

A mild nephrotic syndrome of unknown etiology was diagnosed. After discharge she was followed in the obstetric clinic, where normal blood pressures were found during the pregnancy.

On Aug. 6, 1966, she was readmitted with sharp, pleuritic, lower right chest pain of 3 days' duration radiating to the anterior chest and right shoulder especially on deep breathing. She denied cough or hemoptysis. Blood pressure was 125/65, pulse 100 and regular, temperature 100.4° F., and breathing rate 20 per minute. Decreased breath sounds, dullness to percussion, and pain on pressure were found over the right lower lateral chest. There was no edema of the extremities and Homan's sign was

**Table I.** *Serum electrophoresis*

| *Date* | *Albumin (3.73 ± 0.21)* | *$\alpha_1$-globulin (0.21 ± 0.05)* | *$\alpha_2$-globulin (0.59 ± 0.12)* | *$\beta$-globulin (0.98 ± 0.23)* | *$\gamma$-globulin (0.82 ± 0.14)* |
|---|---|---|---|---|---|
| 6/ 3/66 | 2.6 | 0.4 | 1.2 | 1.6 | 0.4 |
| 8/ 8/66 | 2.5 | 0.4 | 1.5 | 1.3 | 0.5 |
| 3/15/67 | 1.1 | 0.3 | 1.6 | 0.9 | 0.3 |
| 5/ 3/67 | 1.8 | 0.2 | 1.3 | 1.0 | 0.1 |
| 5/ 8/67 | 1.5 | 0.5 | 1.8 | 1.1 | 0.2 |
| 5/23/67 | 1.5 | 0.2 | 1.4 | 0.7 | 0.5 |
| 6/11/67 | 1.9 | 0.2 | 1.2 | 0.9 | 0.4 |

negative. Chest x-rays showed right pleural effusion. Abnormal laboratory findings included a white count of 11,650 with 84 percent neutrophils, an erythrocyte sedimentation rate of 119 per 60 minutes, urine protein 3.7 Gm. per 24 hours, Addis count $7.6 \times 10^6$ casts, most finely and coarsely granular. A purified protein derivative test was strongly positive. Platelet count, BUN, creatinine clearance, lactic acid dehydrogenase and bilirubin were normal.

The initial diagnosis was pulmonary tuberculosis; multiple pleural fluid, urine, and gastric cultures were obtained, and therapy with para-aminosalicylic acid and isonicotinic acid hydrazide was started. The patient then developed left leg pain and a positive Homan's sign as well as left pleuritic chest pain. Chest x-ray revealed a pleural effusion on the left side. Decreased perfusion of the right lower lung field was found on lung scan. Pulmonary embolism was now seriously considered. Hence, PAS was discontinued and the patient was placed on Coumadin, 10 to 15 mg. per day, with prolongation of prothrombin times ranging from 13.5 to 18.3 seconds (control 11.1 to 11.3). On this regimen radiographic chest findings rapidly cleared. She was discharged Sept. 3, 1966, on 10 mg. Coumadin per day. Prothrombin times during follow-up in the outpatient department ranged from 13.5 to 18 seconds (control 11 to 12).

The patient was admitted for the third time March 13, 1967, with shortness of breath, chest pain, and streaks of blood in the sputum. Breath sounds were decreased and dullness increased to percussion at both lung bases. A grade 2/4 pulmonic ejection murmur was noted. Edema of the extremities was not apparent. Except for 4+ protein, urinalysis was normal. Blood pH 7.4, $pO_2$ 71 mm. Hg. and $pCO_2$ 35 mm. Hg. Twenty-four hour urinary proteins ranged from 7 to 12 Gm. Abnormal serum electrophoresis patterns (Table I) and marked hyperfibrinogenemia were again observed. Prothrombin time was 15.1 (control 11.4). Platelet count, serum bilirubin, and L.E. clot test were normal.

On the day after admission sudden onset of severe left-sided pain and increase in dyspnea were noted. Lung scan showed filling defects in both lung bases. An inferior vena cava gram was normal; however, a pulmonary arteriogram revealed occlusion of the right lower lobe pulmonary artery.

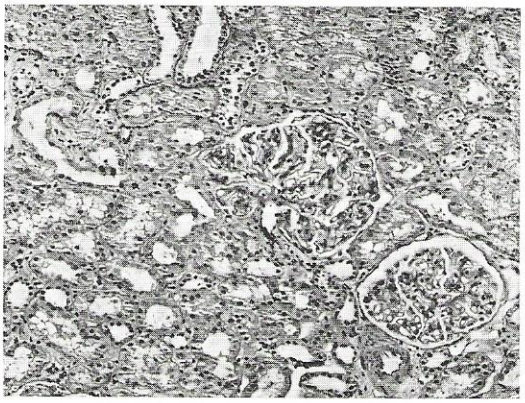

**Fig. 1.** Section of kidney biopsy showing a focal thickening of the glomerular basement membranes throughout the epithelial tufts, characteristic for membranous glomerulonephritis. In addition, occasional small nests of lymphocytes were present in the interstitial tissue not visible in the photograph.

The dyspnea ameliorated rapidly under vigorous heparin therapy. Inferior vena cava ligation was considered but rejected because of an inadequate response to the administration of anticoagulants. Instead vigorous heparin therapy was decided upon. After 12 days heparin was discontinued and the patient was begun and maintained on Coumadin therapy, 20 and 25 mg. on alternate days. After the pulmonary status had stabilized the patient was started on prednisone (100 mg. every other day) for treatment of the nephrotic syndrome.

Discharged on April 12, 1967, the patient returned on May 5, because of chest pain and shortness of breath. Two weeks prior to this admission in the outpatient department. On her last out- the patient had been placed on birth control pills patient department visit on May 3, her prothrom-

bin time exceeded 60 seconds, and Coumadin therapy was therefore temporarily discontinued.

Heparin therapy was reinstituted, and inferior vena cava ligation performed by the transabdominal route. Both ovarian veins and fallopian tubes were ligated and divided. Both renal veins were explored and found to be patent. An open biopsy was obtained from the right kidney. The patient recovered uneventfully and was discharged on June 1, 1967, free of complaints and without pedal edema.

Biopsy showed membranous glomerulonephritis (Fig. 1) which is currently being treated with prednisone and Imuran. The patient continues to take Coumadin, 20 and 25 mg. on alternate days, thereby maintaining the prothrombin time between 19 and 25 seconds (control 11 to 12). In addition she wears elastic supports on both legs and thromboembolic phenomena have not recurred.

## METHODS AND MATERIALS

Fresh, human plasma samples collected by the blood bank served as control studies. Blood samples were obtained from the patient described and were allowed to clot. The serum was collected and quick frozen.

Human plasmin was prepared from human euglobulin and activated with streptokinase (Varidase, Lederle). Previously the amount of streptokinase which provided optimum activity following 30 minutes' incubation at 25° had been determined. The plasmin formed was precipitated at pH 2 with 1M NaCl, as described by Troll and Sherry,[31] dissolved and dialyzed according to Norman[23] against frequent changes of 0.0025N HCl at 0° C. Insoluble matter was removed by centrifugation and the caseinolytic activity of 0.5 or 1 ml. aliquots of the solution determined. Plasmin activity was determined by a modification of the caseinolytic technique described by Remmert and Cohen.[26]

The antiplasmin activity of plasma was determined by the technique described by Shamash and Rimon.[27]

Cellulose acetate microzone electrophoresis was carried out at room temperature for 20 minutes at 250 v. in a Beckman microzone cell with a Duostat power supply and the Beckman $B_2$ buffer ($\mu$, 0.075; pH, 8.6). Proteins were stained with Ponceau red.

Laurell's[16] method of quantitative immunoelectrophoresis in a 1 percent antibody-containing gel was used to determine the amount of alpha$_2$-macroglobulin in normal and the patient's serum. Five microliters of the eight times diluted sera was applied to the wells for a period of 3½ hours at a potential of 7 v. per centimeter. The 1 mm. slide layer of 1 percent (W/V) agarose gel made up in barbital buffer of pH 8.2 ($\mu$ = 0.05) contained 1 percent antiserum.

**Coagulation studies.** Methods used for coagulation studies have been previously described by Edson and co-workers.[5, 6] Fibrin-

**Table II.** *Coagulation studies*

| *Description* | *Date* | | |
|---|---|---|---|
| | *3/16/67* | *5/10/67* | *5/19/67* |
| One stage prothrombin time | 14.9 (13.8)* | 11.9 (13.9) | 12.6 (13.3) |
| Kaolin partial thromboplastin time | 40.4 (37.1) | 24.66 (36.8) | 21.6 (36.3) |
| Thrombin time | 60 (14.9) | 17.0 (13.5) | 17.3 (14.5) |
| Factor II | 100% | 160% | 154% |
| Factor V | 145% | 140% | 122% |
| Factor VII | 115% | 290% | 216% |
| Factor VIII | 305% | 315% | 465% |
| Factor IX | 80% | 150% | 182% |
| Factor X | 100% | 160% | 146% |
| Factor XI | 100% | 136% | 118% |
| Factor XII | 72% | 100% | 78% |
| Fibrinogen | | | 540 mg. %† |
| Euglobulin clot lysis time | | | 5 hours |

*Control values in parentheses.

†Cryofibrinogen present but not quantitated.

ogen was determined by the method of Jacobsson.[14]

## RESULTS

The results of the clotting studies were markedly abnormal (Table II). Factors V and VIII and fibrinogen levels in blood samples obtained prior to heparin administration were elevated. A prolonged thrombin time found on March 16, 1967, probably resulted from heparin therapy. On May 20, and May 29, 1967, the vitamin K–dependent factors were found to be elevated, as were factor VIII and fibrinogen. On May 19, factors VII, VIII, and IX and fibrinogen were affected. A euglobulin clot lysis time of over 5 hours was found.

At no time was free plasmin or increased activator activity observed in this patient's plasma. In addition the fast antiplasmin activity in the patient's serum was two to three times the normal control value. In contrast the slow, progressive antiplasmin activity was only slightly elevated (Fig. 2).

The patient was also resistant to anticoagulant therapy. Large heparin and Coumadin doses were required to obtain adequate anticoagulation. Unfortunately no available methods can quantitate this reaction more precisely.

Abnormalities in the plasma protein concentrations were alluded to in the case report. Data on plasma electrophoresis are summarized in Table I. A greatly decreased gamma globulin concentration, hypoalbumenemia and a consistently elevated alpha$_2$-globulin level were found. These abnormalities initially responded to steroid therapy but then rapidly returned to pretreatment levels.

The increase in the alpha$_2$-globulin fraction (Table I and Fig. 3) was the result of an increase in alpha$_2$-macroglobulin as determined by the Ouchterlony plate tech-

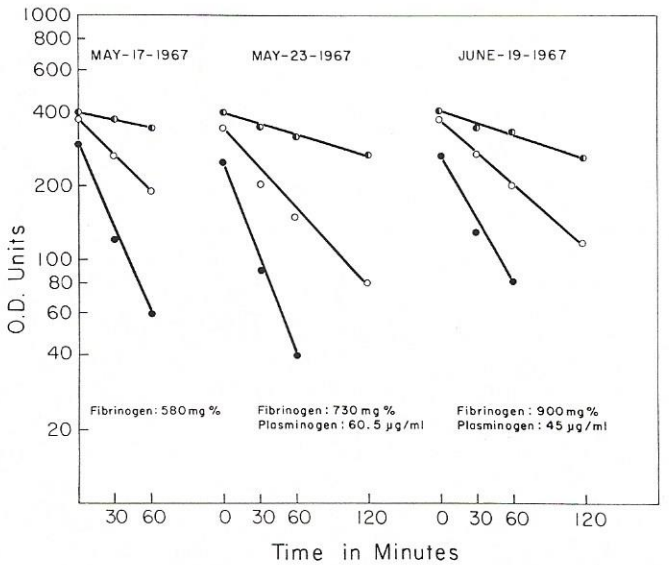


**Fig. 2.** Antiplasmin assays and plasma fibrinogen and plasminogen values. Incubation of plasmin with buffer (O——O), normal plasma (◒——◒), and patient's plasma (●——●) at 37° C. followed by periodic testing of aliquots of each mixture for residual plasmin activity provided the data for these plasmin disappearance curves. Fast or $\alpha_2$-antiplasmin represents the difference in residual plasmin activity at zero time between a buffer control and plasma; it is greatly increased in the patient's plasma. The concentration of slow or $\alpha_1$-antiplasmin is derived from the determination of the residual plasmin activity every 30 minutes for 1 to 2 hours. This activity in the patient's plasma was not significantly different from that of normal plasma. In addition fibrinogen levels were elevated (normal values 300 ± 100 mg. percent) more than plasminogen values (normal values 35 ± 10 µg per milliliter).

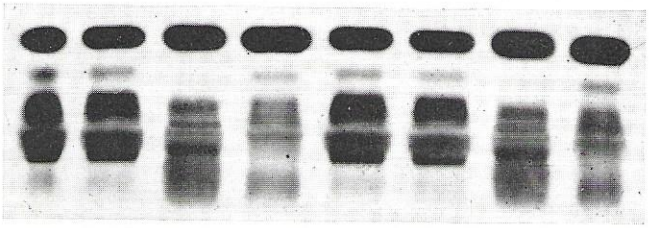

**Fig. 3.** Electrophoresis of different sera on cellulose acetate. From left to right: 1, 2, 5, and 6, patient's serum; 3 and 7, $\alpha_1$antitrypsin deficient serum; 4 and 8, normal human serum. Note the marked increase in $\alpha_2$-globulin concentration in the patient's serum.

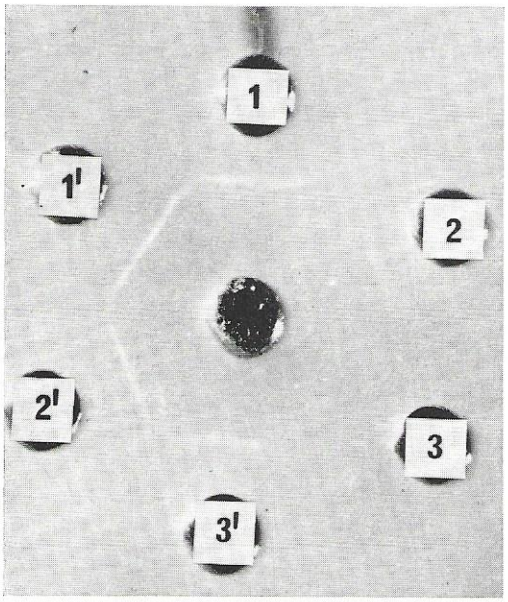


**Fig. 4.** Quantitative determination of $\alpha_2$-macroglobulin according to the method of Ouchterlony. 0: Rabbit antihuman serum; *1*, *2*, and *3*: normal human serum (1/16, 1/32 and 1/64 dilution); *1'*, *2'*, and *3'*: patient's serum (1/16, 1/32, and 1/64 dilution).

nique when precipitant bands of dilutions of the patient's plasma against alpha$_2$-macroglobulin were compared to those obtained with control plasma (Fig. 4) and by a more direct quantitative immunodiffusion technique previously described by Laurell[16] (Fig. 5).

## DISCUSSION

A patient with nephrotic syndrome and marked abnormalities in the serum electrophoretic pattern experienced recurrent thromboembolic episodes. Response to anticoagulant therapy was far from consistent and only rarely was a prothrombin time value within therapeutic range obtained. Prior to the last hospital admission, the patient was started on an oral contraceptive drug that has been implicated in thrombogenesis in some individuals. Also anticoagulant therapy was discontinued when the prothrombin time was found to be greatly prolonged immediately prior to her last admission. In the presence of normal liver function it was assumed that the prothrombin prolongation resulted from either an excess of anticoagulants or increased prothrombin consumption, which is observed in extensive thrombosis.

Because the patient was originally admitted with symptoms of thromboembolism before she had received Enovid and before anticoagulant therapy was abruptly discontinued, an inherent clotting defect was assumed to exist.

The initial episode probably represented the development of a postpartum thrombophlebitis. Damage to the pelvic and leg veins was apparently severe enough to contribute to recurrent thrombotic episodes, especially in a patient who occasionally developed hypercoagulability of the blood as suggested by a markedly shortened kaolin partial thromboplastin time on two of the three occasions when this test was performed.

Thus, two factors are implicated in the development of the patient's venous thrombosis: an altered vessel wall and a hypercoagulability state. The latter, according to

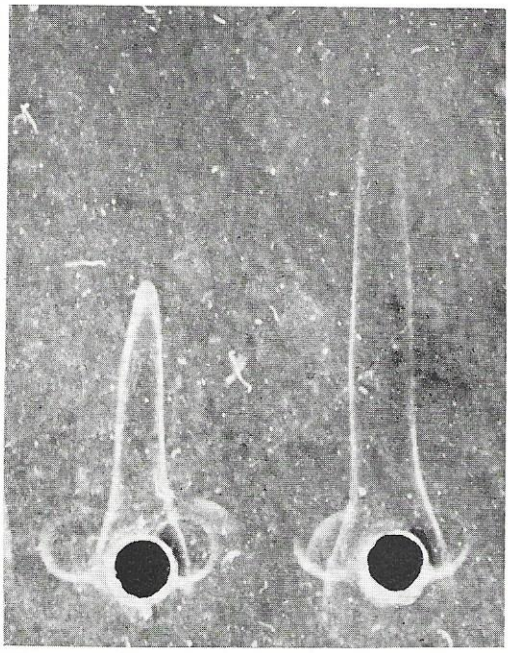

Fig. 5. Quantitative determination of the $\alpha_2$-macroglobulin according to the method of Laurell.[16] Left: normal human serum; right: patient's serum (both diluted eight times). Since the protein concentration is proportional to the surface area underneath the curve, it is apparent that the patient's serum contains approximately twice the $\alpha_2$-macroglobulin concentration of normal human serum.

Antley and McMillen,[1] is usually associated with an enhanced turnover rate of clotting factors. As a result their concentration in plasma would be decreased. In contrast, in the present case not only fibrinogen but also factors V, VII, VIII, IX, and X were markedly elevated. These results, in the presence of increased turnover rates, suggest that the production of these coagulation factors was also greatly enhanced. Accelerated production rates for K dependent factors (VII, X, and II) would result in increased requirements for vitamin K antagonists, such as Coumadin, in order to elicit a depression of prothrombin production consistent with adequate anticoagulation. Such an increased anticoagulant requirement was found on several occasions, but even when higher doses of Coumadin were used the response remained erratic. This unusual form of anticoagulant resistance eventually necessitated surgical intervention.

Deep venous thrombosis in the lower limb is not an uncommon problem, especially in postoperative and postpartum patients. Medical therapy for deep vein thrombosis usually prevents the immediate complications of pulmonary emboli. According to Nussbaum and Blakemore,[25] however, there remains a failure rate of 5 to 10 percent with anticoagulant therapy.

Thrombosis or thromboembolism in the nephrotic syndrome may moreover be somewhat more common than has been appreciated. Thrombosis of the vena cava and hepatic veins, as noted by Dodds and associates,[4] of the pulmonary arteries noted by Levin and associates,[17] Symchych and Perrin,[29] and Gootman and associates,[13] and of the femoral artery observed following femoral venipuncture by Goldbloom and Hillman[12] have all been reported in association with nephrotic syndrome.

Focal thickening of the glomerular basement membrane, indistinguishable from that noted in our patient, was previously observed by McKay[18] during or shortly after intravascular coagulation. The thickening consisted of deposition of granular material in the basement membrane. When studied by immunochemical techniques shortly after a defibrination episode, this material was indistinguishable from fibrinogen. It probably represents partially polymerized fibrin, a material previously noted in homografted kidneys during chronic rejection. The long-term symptoms suggested that it had been present so long that an attempt to demonstrate residual fibrin in the renal biopsy was not warranted. Its relationship to fibrin was therefore not established. The possibility remains, however, that the initial lesion resulted as a consequence of intravascular coagulation.

Other factors implicated in the thrombogenic tendencies of this patient are related to the hyperfibrinogenemia and marked increase in fast antiplasmin activity.

The significance of hyperfibrinogenemia in nephrosis was first observed by Takeda

and Chen,[30] and its relationship to thromboembolic phenomena was explored by Bang and co-workers[2] who found that thrombi, formed in the presence of hyperfibrinogenemia, are very resistant to the action of fibrinolytic enzymes.

Besides evidence for hypercoagulability of blood, as manifested by a shortened partial thromboplastin time, a prolonged euglobulin clot lysis time was found, indicating hypofibrinolysis. Hypofibrinolysis promotes thrombosis when it results from increased antiplasmin activity of the blood, regardless of whether the increased activity resulted from natural[20-22] or from synthetic antiplasmin.[3, 8]

Two antiplasmins in human plasma and serum have been described by Norman.[24] A heat labile $alpha_1$-globulin is a competitive inhibitor, whereas the more heat stable $alpha_2$-antiplasmin reacts in a bimolecular reaction and proceeds at a fast rate. The latter, which represents less than one fourth of the total antiplasmin in activity of plasma, nevertheless appears to be the more important inhibitor, as we recently demonstrated in a group of patients with greatly enhanced fibrinolytic activity.[10] These patients rarely showed free plasmin activity. Their antiplasmin activity was normal. They demonstrated a marked decrease in fast antiplasmin that paralleled the fall in the plasminogen level. This suggested that the loss of antiplasmin activity resulted from plasmin neutralization that accompanied plasminogen activation.

Immunochemical techniques have permitted the recognition of at least five different $alpha_2$-globulins as distinctly separate entities. This case report of a young woman with nephrosis shows that the elevation of $alpha_2$-globulin found on cellulose acetate electrophoresis resulted from marked, increased $alpha_2$-macroglobulin levels, confirming a recent observation made by Steines and Mehl.[28] The nephrosis that accompanies renal vein thrombosis induces changes in the protein composition of serum identical to those observed and described for this patient.[9] Hence the serum of patients with suspected renal vein thrombosis should be examined by current electrophoretic techniques for increases in the $alpha_2$-globulin fraction. This will serve as a simple, rapid, and valuable diagnostic test.

In addition to elevated $alpha_2$-macroglobulin levels, the fast or $alpha_2$-antiplasmin activity in our patient's plasma was found to be increased. It paralleled the increase in $alpha_2$-macroglobulin concentration. Mehl and associates[19] have shown that the $alpha_2$-macroglobulin causes trypsin binding, and Lanchantin and co-workers[15] have shown that this fraction causes antithrombin clotting activity. The findings obtained in this patient suggest that the $alpha_2$-macroglobulin represents the fast or $alpha_2$-antiplasmin activity as well.

Recently we observed that $alpha_1$-antitrypsin represents an antiproteinase with a wider range of activity than its name would suggest.[11] It is possible, as Ganrot[7] suggested, that $alpha_2$-macroglobulin also causes a wide range of protease inhibition.

## SUMMARY

Recurrent episodes of pulmonary emboli were observed in a young woman with nephrosis. Quantitative abnormalities in the plasma proteins were decreased albumin and gamma globulin and increased $alpha_2$-globulin concentration.

Several clotting proteins were found to be increased despite the enhanced turnover rate that is associated with hypercoagulability of blood. This interesting and unusual set of circumstances probably led to an erratic anticoagulant response that ultimately required inferior vena cava ligation to prevent further recurrence of pulmonary emboli.

The elevated $alpha_2$-globulin levels could be attributed to increase in the $alpha_2$-macroglobulin fraction which also closely paralleled the increase in the fast or $alpha_2$-antiplasmin activity of the patient's blood. This suggests that the fast antiplasmin is identical with the $alpha_2$-macroglobulin fraction of plasma or serum.

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