PLASMA-EXCHANGE IN THE TREATMENT OF CRYOGLOBULINEMIA *

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Cryoglobulinemia is a clinical and biochemical abnormality that was first described by WINTROBE and BUELL[16] in 1933. Since then many cases have been reported[1,3,5,10,12], and the past several decades have witnessed striking advances in the understanding of this disease[6,7]. Contrary to the explosive growth of knowledge regarding the pathogenesis of cryoglobulinemia, advances in treatment have been less spectacular. Steroids and cytotoxic drugs administered at high doses or in intermittent short courses have not had much success in many cases. Other contributions have been made with the introduction of plasma-exchange (PE), though the results are contrasting[2,4,11].

In this paper, findings are described of 10 patients with mixed cryoglobulinemia (MCG) in whom the effects of short-term PE, combined with steroids and/or cytotoxic drugs, were studied.

MATERIALS AND METHODS

Patients - Ten patients with MCG, aged 33 to 62, were chosen for treatment. Details of the clinical signs and treatment regimen are reported in tab. 1. Drug therapy for all patients consisted of steroids and/or cytotoxic drugs in the first stage. This therapy was modified by the attending physician only in response to evidence of changes in disease activity. Patients considered to have active MCG at the time of presentation received 1

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patient	age	clinical features on admission	previous treatment	therapy administered
V.M.	47	arthralgias, purpura	prednisone 10 mg/day	azathioprine 150 mg/day prednisone 25 mg/day
M.M.	62	arthralgias, Raynaud's phenomenon	non-steroid anti-inflammatory drugs	prednisone 10 mg/day azathioprine 150 mg/day
L.G.	45	hypertension, purpura, hepatitis, nephritis, fever, anemia	none	prednisone 25 mg/day azathioprine 150 mg/day
D.S.	33	edema, purpura, anemia	non-steroid anti-inflammatory drugs	none
D.M.	52	edema, nephritis, purpura	methylprednisolone 8 mg/day	methylprednisolone 8 mg/day
V.A.	46	arthralgias, papules, hepatitis, pulmonary fibrosis	prednisone 10 mg/day	prednisone azathioprine 150 mg/day
C.C.	43	purpura, arthralgias, nephritis, hypertension, fever	prednisone 50 mg/day	methylprednisolone 1 g daily x 3 prednisone 50 mg/day azathioprine 150 mg/day
A.G.	49	purpura, nephritis, edema, hypertension	prednisone 10 mg/day	prednisone 5 mg/day
C.V.	60	hepatitis	none	none
P.G.	42	purpura, nephritis, hypertension	prednisone 10 mg/day	methylprednisolone 1 g daily x 3 prednisone 100 mg/day

Tab. 1 - Clinical signs and treatment regimen of the patients considered.

mg/kg/day of prednisone for one month with or without high dose methylprednisolone pulses (1 g/day) for 3 days. If the clinical conditions continued to deteriorate, PE was started.

Seven out of 10 patients had renal involvement; in fact, renal biopsy with light and fluorescent microscopy showed membranoproliferative glomerulonephritis in 4 cases and mesangial proliferative glomerulonephritis in 3 cases.

Plasma-exchange - PE was performed by means of an intermittent flow centrifuge (Haemonetics mod. 30). During each session, large or small disposable bowls were used, depending on the plasma volume of the patient. This parameter was calculated on the basis of the hematocrit and body weight. In each session 50% of the effective plasma was exchanged. Plasma was replaced by different solutions: over the years the choice of solutions has varied. Before 1981, plasma was replaced by 50% normal saline and 50% fresh frozen plasma or Hemagel and serum human albumin at 5%, when fresh frozen plasma was not present in the blood bank. After 1981, with the establishment of the Italian Hemapheresis Committee[9], it was decided to replace plasma by normal saline (25%), Hemagel (25%), human serum albumin at 5% (25%) and, finally, by fresh frozen plasma.

The protocol of the PE for the first 2 months consisted of one session daily for 3 days or one session on alternate days for 2 weeks, then two sessions weekly for 2 weeks and, finally, one session weekly for one month.

Methods - The blood parameters selected for monitoring the disease during PE were the cryoglobulins and serum levels of some complement components such as C4, C3 and properdin factor B. In patients with renal damage, serum creatinine and daily proteinuria were measured during the course of the disease.

The cryocrit was determined in 20 ml of blood obtained without anticoagulant, using sterile plastic syringes prewarmed to 37 °C. Each blood sample was left to clot at 37 °C for 3-4 h, the serum was separated by centrifugation and then kept at 4 °C for 5 days. When a cryoprecipitate had formed, it was separated by centrifugation and washed three times in phosphate-buffered saline at 4 °C. The protein concentration of cryoglobulins was evaluated by the biuret method on an aliquot of cryoprecipitate dissolved in glycine-HCl buffer 0.1 M, pH 3.7, and was expressed as the concentration present in the original volume of serum. The composition of cryoglobulins was determined by immunoelectrophoresis, using commercially available antiserum against whole human serum, and monospecific antisera to IgG, IgA, IgM, λ and $\varkappa$ chains (*Behring Institute*).

Serum complement levels of C4, C3 and properdin factor B were evaluated by radial immunodiffusion, as described by MANCINI et al.[8], using monospecific antisera incorporated in 1.5% agarose containing Veronal buffer 0.0075 M, pH 8.6, and EDTA in a final concentration of 0.01 M. Values were expressed as a percentage of pooled normal sera (% PNS) taken from healthy blood donors. The normal mean values were 108.6 $\pm$ 3.6 (SEM) for C4, 100.5 $\pm$ 3.3 for C3 and 96.9 $\pm$ 2.3 for properdin factor B.

RESULTS

Cryoglobulins detected in 10 patients were composed of IgG-IgM in 7 patients (5 of which had renal involvement), IgG-IgA in one case, IgG-IgM-IgA in one case and IgG-IgM-C1q in the last case.

There were different types of response to PE. The findings obtained can be divided into 4 specific groups, represented by the following 4 cases (figs 1-4).

D.S., a 32-year-old woman, had a history of anemia, purpura and arthralgias since 1975. After 4 years of recurrent purpura, MCG was diagnosed. Characterization of the cryoglobulin showed IgG and IgA. In December 1979 she showed hematuria and proteinuria, and in February 1980 she began PE. The patient received no other treatment at the time of PE. Intensive PE (3 sessions per week) produced a rapid decrease in cryoglobulin levels and a marked improvement in the symptoms. When the frequency of sessions was reduced to one per week, cryoglobulins rose, accumulating rapidly at the end of PE. Proteinuria was reduced during intensive PE, but recurred within 4 months when the frequency of sessions was reduced. After PE therapy the patient had an episode of non-A non-B hepatitis.

L.G., a 46-year-old woman, had a history of hypertension, anemia, purpura and chronic hepatitis, with considerable splenomegaly. After 3 years of recurrent purpura, MCG was diagnosed. Cryoglobulin analysis revealed IgM ($\varkappa$) and IgG. HBsAg was negative. Because of proteinuria and decreasing renal function, she was started on prednisone (25 mg/day) in July 1981. Prednisone was reduced to 12.5 mg/day after 2 months of therapy, because of

the improvement achieved (a reduction in serum creatinine and daily proteinuria); in contrast, high levels of cryoglobulins persisted. Blood pressure was 220/130 mmHg and did not respond to a wide range of antihypertensive drugs. C3 and C4 fluctuated at low levels. Eight months later, because of lack of response, azathioprine and PE were started. One month after the initiation of therapy the purpura disappeared, cryoglobulins decreased and blood pressure fell to 180/90. PE was stopped after one month, while prednisone and azathioprine were continued. PE was followed by a rapid and prolonged clinical improvement, but drug therapy did not prevent an increase in cryoglobulins.

V.M., a 46-year-old woman, had a history of progressive purpura, arthralgias and Raynaud's phenomenon of the extremities. After 3 years of recurrent purpura, MCG was diagnosed and analysis of the cryoglobulins showed IgG-IgM-C1q. A renal biopsy revealed membranoproliferative glomerulonephritis. In July 1981, therapy with prednisone (25 mg/day) and azathioprine (150 mg/day) was begun but, as arthralgias persisted and intensive purpura and Raynaud's phenomenon recurred, PE was initiated. During the initial course of PE there was a fall in cryoglobulins, with an improvement in the clinical condition. After suspension of PE, azathioprine controlled the increase of cryoglobulins for 9 months. A deterioration in the clinical picture began in March 1982, and she started a second course of PE for one month, which did not produce a decrease in cryoglobulins. On the other hand, the purpura and arthralgias disappeared.

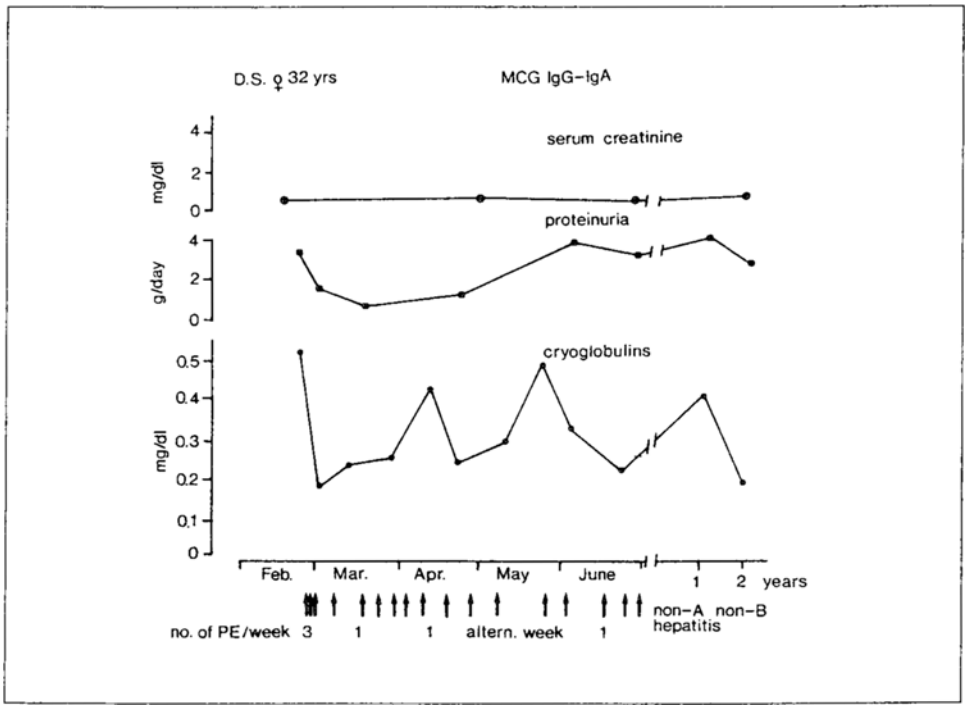

Fig. 1 - Response to plasma-exchange in patient D.S.

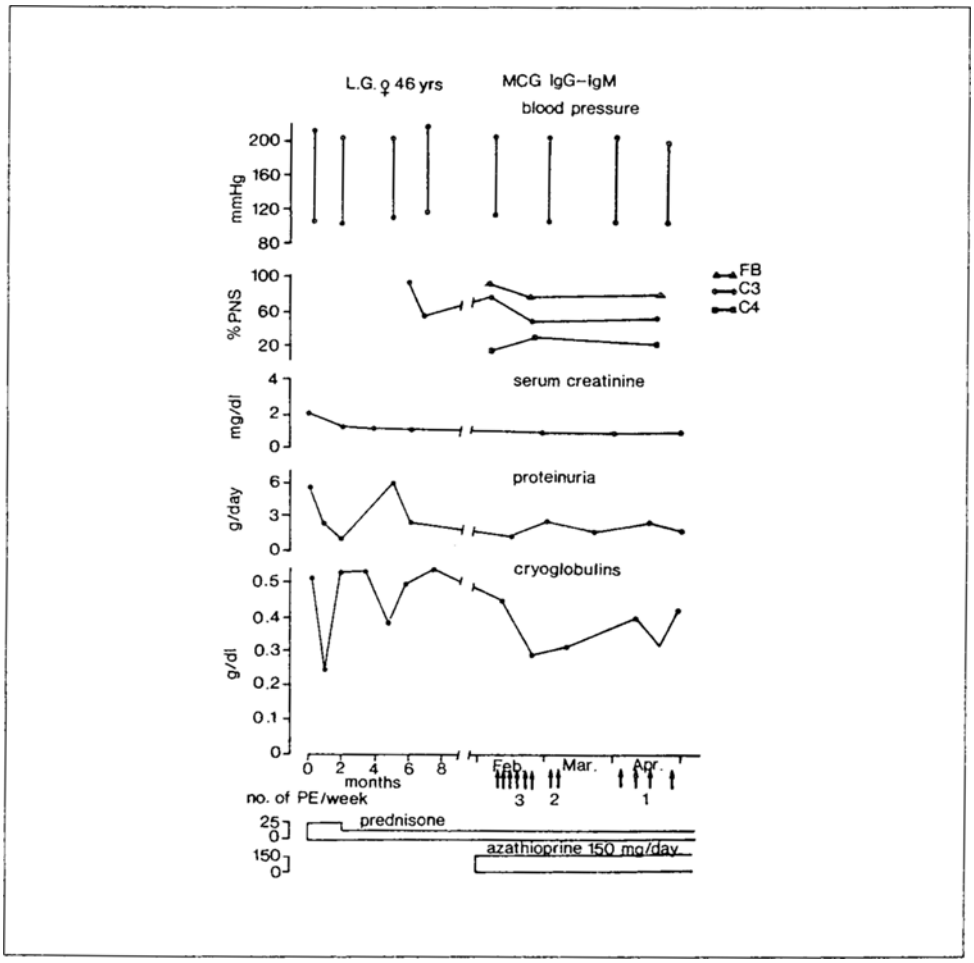

Fig. 2 - Response to plasma-exchange in patient L.G.

M.M., a 62-year-old man, had a history of arthralgias and purpura, paraesthesia and very cold feet since 1977. The course of the disease was characterized by Raynaud's phenomenon and recurrent episodes of purpura. In November 1981, he was admitted to hospital with diffuse purpura of the extremities and arthralgias. Cryoglobulins of type IgG-IgM (κ) were found. He was treated with non-steroid anti-inflammatory drugs, but his condition deteriorated and cytotoxic drugs, with PE, were administered. During PE, the level of the cryoglobulins fell, but when the frequency of sessions was reduced, the cryoglobulins increased. Although intensive PE, combined with prednisone and azathioprine, caused a rapid decrease in cryoglobulins, his condition continued to deteriorate and he developed gangrene of two fingers on his left hand.

The limits of PE in MCG were studied by measuring circulating cryoglobulins before and after each session for a total of 29 sessions. PE can remove 30% of circulating cryoglobulins when 50% of plasma is exchanged.

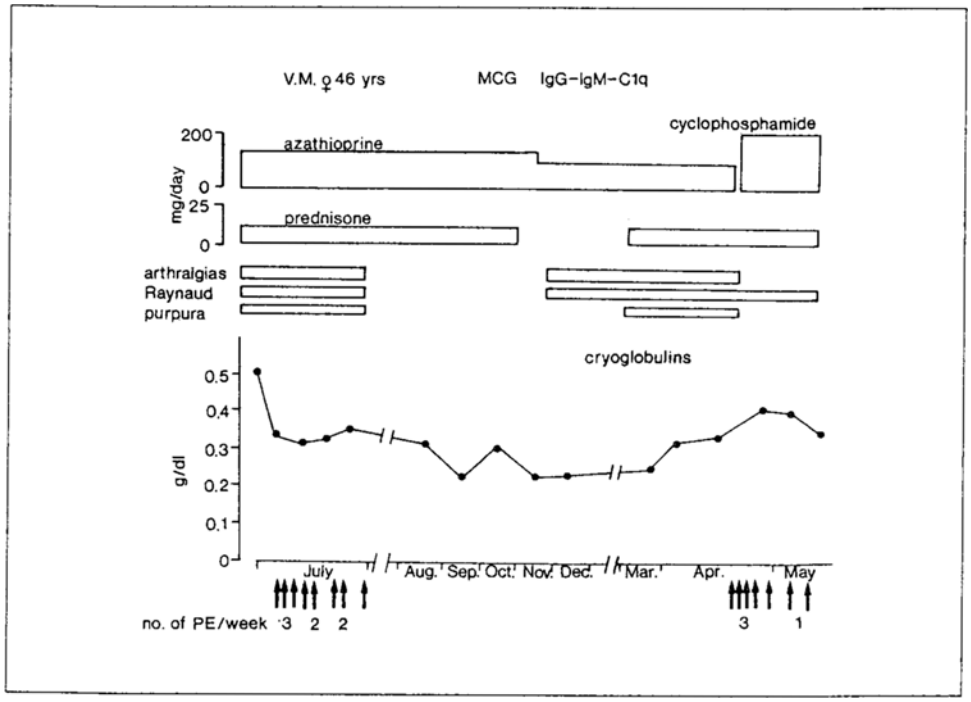

Fig. 3 - Response to plasma-exchange in patient V.M.

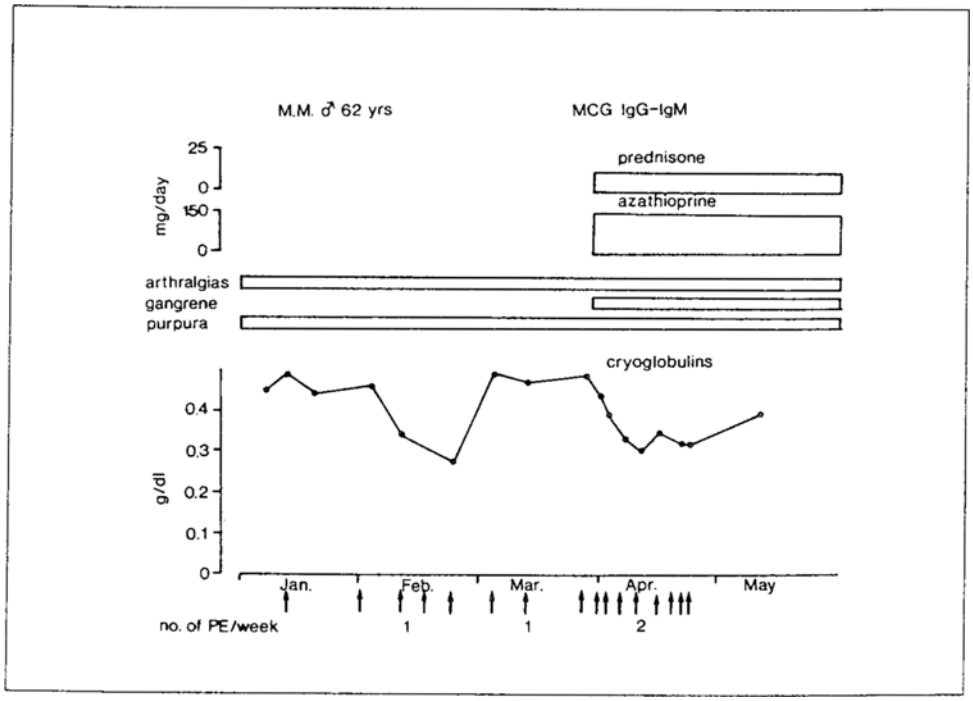

Fig. 4 - Response to plasma-exchange in patient M.M.

This low percentage was confirmed when the residual mean percentage of cryoglobulins was measured during 20 regular PE sessions. Cryoglobulins reached a plateau of 45% after only 15 sessions. These findings confirm that PE therapy removes only a low percentage of cryoglobulins from the blood.

DISCUSSION

In this study different responses to PE in patients with MCG are reported.

The first group shows that the use of PE alone in the treatment of MCG might be of value as an investigative tool, but it is potentially hazardous, and could lead to clinical deterioration during the period of rebound. The same results have been reported by VERRIER JONES et al.[15] and by SCHLANSKY et al.[14] in patients with systemic lupus erythematosus. None of the patients treated with PE alone showed an improvement in clinical condition, and cryoglobulins returned to pretreatment levels within 2-3 weeks of PE, with an increase in proteinuria in those with glomerulonephritis.

In the second group it was demonstrated that PE may be a valuable additional form of therapy in MCG with hypertension, but when the frequency of PE is reduced, elevated levels of cryoglobulins appear. Azathioprine does not suppress the rebound phenomenon.

Intensive PE does not always cause a reduction in cryoglobulin levels in symptomatic patients, as shown in the third group. In fact, when PE was started again, cryoglobulins rose even when administering prednisone and cyclophosphamide. This finding was also seen in patients of the fourth group, in whom PE was not followed by an improvement of the clinical symptoms, even when intensive PE was combined with cytotoxic drugs. The clinical success of the procedure probably depends on the rate of cryoglobulin synthesis and on the percentage of removal. The precipitation of cryoglobulins at room temperature during the extracorporeal procedure could also have lowered the efficacy of the PE. The results show that adequate PE can remove a low percentage of circulating cryoglobulins, and PE is more effective in patients with systemic lupus erythematosus, where 80% of circulating immune complexes can be removed during intensive PE[13].

On the basis of this experience, it is thought that the least possible intervention should be made. It is suggested that non-steroid anti-inflammatory drugs, or low doses of steroids should be used when cutaneous vasculitis and arthralgias are present. High dose methylprednisolone pulses associated with immunosuppressive drugs are indicated in cases with renal involvement and/or extensive vasculitis and/or neurological manifestations. Adjunctive PE should be restricted to those patients who do not respond to aggressive chemotherapy.

SUMMARY

Plasma-exchange was used in 10 patients with mixed cryoglobulinemia. The procedure was used as a primary therapeutic tool to reduce cryoglobulin levels, and in combination with prednisone and cytotoxic drugs. The results show that PE alone is detrimental, although it may be an important adjunct to conventional therapy in MCG with progressive deterioration

in the clinical condition, and in those patients who do not respond to drug therapy alone. The weak response to PE in MCG may be due to many technical variables, but mainly to the low percentage of circulating cryoglobulins removed.

REFERENCES

1. ADAM C., MOREL-MAROGER L.: Cryoglobulins in glomerulonephritis not related to systemic disease · Kidney int. *3*, 334, 1973.
2. BERKMAN E. M., ORLIN J. B.: Use of plasmapheresis and partial plasma exchange in the management of patients with cryoglobulinemia · Transfusion (Philad.) *20*, 171, 1980.
3. BONOMO L., DAMMACCO F.: Immune complex cryoglobulinemia in lepromatous leprosy · Clin. exp. Immunol. *9*, 175, 1971.
4. GELTNER D., KOHN R. W., GOREVIC P., FRANKLIN E. C.: The effect of combination therapy (steroids, immunosuppressive, and plasmapheresis) on 5 mixed cryoglobulinemia patients with renal, neurologic, and vascular involvement · Arthr. and Rheum. *24*, 1121, 1981.
5. GOREVIC P. D., KASSAB H. J., LEVO Y., KOHN R., MELTZER M., PROSE P., FRANKLIN E. C.: Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients · Amer. J. Med. *69*, 287, 1980.
6. GREY M. M., KOHLER P. F.: Cryoimmunoglobulins · Semin. Haemat. *10*, 87, 1973.
7. LEVO Y., GOREVIC P. D., KASSAB H. J., ZUCKER-FRANKLIN D., GIGLI I., FRANKLIN E. C.: Mixed cryoglobulinemia. An immunocomplex disease often associated with hepatitis B virus infection · Trans. Ass. Amer. Phycns *40*, 167, 1977.
8. MANCINI G., CARBONARA A. O., HEREMANS J. F.: Immunochemical quantitation of antigens by single radial immunodiffusion · Immunochemistry *2*, 235, 1965.
9. MARCONI M., MERCURIALI F., SIRCHIA G.: Il *plasma-exchange* · Edizioni Ortho Diagnostic Systems. Milano, 1981.
10. MCINTOSH R. M., KAUFMAN D. B., KULVINSKAS C., GROSSMAN B. J.: Cryoglobulins. I. Studies on the nature, incidence and clinical significance of serum cryoproteins in glomerulonephritis · J. Lab. clin. Med. *75*, 566, 1970.
11. MCLEOD B. C., SASSETTI R. J.: Plasmapheresis with return of cryoglobulin-depleted autologous plasma (cryoglobulinpheresis) in cryoglobulinemia · Blood *55*, 866, 1980.
12. MELTZER M., FRANKLIN E. C.: Cryoglobulinemia. A study of 29 patients · Amer. J. Med. *40*, 828, 1966.
13. SCHENA F. P., MANNO C., DIMONTE D., CARABELLESE S., CAZZATO L., PAGLIONICO N.: Plasma exchange in systemic lupus erythematosus · J. artif. Org. (In press).
14. SCHLANSKY R., DEHORATIUS R. J., PINCUS T., TUNG K. S. K.: Plasmapheresis in systemic lupus erythematosus. A cautionary note · Arthr. and Rheum. *24*, 49, 1981.
15. VERRIER JONES J., ROBINSON M. F., PARCIANY R. K., LAYFER L. F., MCLEOD B.: Therapeutic plasmapheresis in systemic lupus erythematosus · Arthr. and Rheum. *24*, 1113, 1981.
16. WINTROBE M. M., BUELL M. U.: Hyperproteinemia associated with multiple myeloma. With report of a case in which an extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynaud's disease · Bull. Johns Hopk. Hosp. *52*, 156, 1933.

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