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Plasma levels of granulocyte elastase during hemodialysis: Effects of different dialyzer membranes

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Plasma levels of granulocyte elastase during hemodialysis: Effects of different dialyzer membranes. Plasma levels of granulocyte elastase in complex with α_1-proteinase inhibitor during hemodialysis were investigated in 15 patients (37.4 ± 3.2 years) undergoing maintenance hemodialysis (47.0 ± 12.9 months) with dialyzers made from cellulose hydrate, cuprophan, polymethylmethacrylate, ethylene-vinyl alcohol copolymer, and polyacrylonitrile. Cellulose hydrate membrane caused a maximal increase of the plasma levels of granulocyte elastase in complex with α_1-proteinase inhibitor (E-α_1PI: 1,659.0 ± 256.8 ng/ml). Patients dialyzed with polyacrylonitrile dialyzers failed to exhibit comparable plasma levels of granulocyte elastase (E-α_1PI: 237.8 ± 22.9 ng/ml). During hemodialysis plasma E-α_1PI values rose to a peak 643.0 ± 174.7 ng/ml in patients on polymethylmethacrylate dialyzers, to 557.5 ± 120.0 ng/ml on cuprophan dialyzers, but to only 381.9 ± 54.0 ng/ml on ethylene-vinyl alcohol copolymer dialyzers. Plasma lysozyme levels decreased significantly in the presence of polyacrylonitrile and polymethylmethacrylate membranes. We conclude that the degree of PMNs stimulation depends on the nature of the dialyzer membrane material. The following membranes induce a reaction of increasing intensity: polyacrylonitrile, ethylene-vinyl alcohol copolymer, cuprophan, polymethylmethacrylate, and cellulose hydrate.

Taux plasmatique de l'élastase granulocytaire pendant l'hémodialyse : effets de différentes membranes dialytiques. Le taux plasmatique de l'élastase granulocytaire lié avec l'inhibiteur de α1-protéinase (E-I α1P) a été étudié pendant l'hémodialyse chez 15 patients (âgés de 37,4 ± 3,2 ans) hémodialysés chroniques (depuis 47,0 ± 12,9 mois) avec des dialyseurs à fibres creuses faites d'hydrates de cellulose, de cuprophane, de polyméthylméthacrylate, de copolymèreéthylène-vinyl-alcool et de polyacrylonitrile. La membrane d'hydrate de cellulose a provoqué une augmentation maximale des taux plasmatiques de l'E-Iα1P (1659,0 ± 256,8 ng/ml). Les sujets dialysés avec des dialyseurs en polyacrylonitrile n'ont pas montré des taux plasmatiques comparables (E-Iα1P = 237,8 ± 22,9 ng/ml). Pendant l'hémodialyse, l'E-Iα1P plasmatique a atteint un pic de 643,0 ± 174,7 ng/ml chez les patients dialysés sur polyméthylméthacrylate, de 557,5 ± 120,0 ng/ml sur cuprophane, mais seulement de 381,9 ± 54,0 ng/ml sur copolymèreéthylène-vinyl-alcool. Les taux plasmatiques du lysozyme ont significativement diminué en présence des membranes de polyacrylonitrile et de polyméthylméthacrylate. Nous concluons que le degré de stimulation granulocytaire dépend de la nature de la membrane du dialyseur. Les membranes suivantes produisent une réaction d'intensité croissante : polyacrylonitrile, copolymèreéthylgène-vinyl-alcool, cuprophane, polyméthylméthacrylate, et hydrate de cellulose.

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Transient granulocytopenia occurs in patients during the initial phases of hemodialysis with cellulosic membranes [1–4]. Such leukopenia results from pulmonary sequestration of leukocytes provoked by complement-derived fragments [5–8].

Neutrophil granulocytes contain the neutral proteinases elastase, cathepsin G, and collagenase. The activity of proteases from human polymorphonuclear neutrophils on blood smears was tested during hemodialysis with cuprophan hollow-fiber dialyzers [9–12]. Granulocyte elastase is probably largely responsible for tissue death, both because of its broad substrate specifity and its abundance in the PMN leukocytes. In vitro, it degrades various plasma proteins such as transferrin, immunglobulins, fibronectin, and several clotting factors (F I, III, V, VII, VIII, XII, XIII) (for review, see [13].) Granulocyte elastase and cathepsin G also cause a limited degradation of the third and fifth complement factor of human complement [14, 15]. On the other hand, both C3a and C5a activate granulocytes.

Complement activation was temporally correlated with hemodialysis leukopenia using cuprophan hollow-fiber dialyzers. By contrast, patients dialyzed with polyacrylonitrile dialyzers failed to exhibit hemodialysis leukopenia and displayed only very modest increases in their plasma C3a levels [16]. Furthermore, during hemodialysis, plasma C3a rose from 401 to 6,325 ng/ml on cuprophan dialyzers, but from 426 to only 3,637 in patients on cellulose acetate devices [17].

We have, therefore, attempted to characterize the changes in plasma levels of granulocyte elastase in patients undergoing maintenance hemodialysis, with particular reference to the effects of cellulosic membranes and with noncellulosic membrane formations, which have been reported to be more compatible with blood [3, 4, 16–18].

Methods

Patients

Fifteen chronically uremic patients (7 male and 8 female), aged 57.4 ± 3.2 years (mean ± SEM, range 26 to 79) undergoing regular hemodialysis treatment (RDT) for 47.0 ± 12.9 months (range 6 to 132) were studied. Hemodialysis was performed 11.3 ± 0.3 hr weekly using five different dialyzers in ten patients and three different dialyzers in five patients. Each patient served as his or her own control. The primary kidney disease was chronic glomerulonephritis in seven cases, polycystic kidney disease in two cases, diabetic glomerulosclerosis in three cases, interstitial nephritis in two cases, and tumor nephrectomy in one case.

Predialysis plasma concentration of creatinine was 11.6 ± 0.98 mg/dl and of urea, 158.4 ± 12.4 mg/dl. Patients were each dialyzed with dialyzers made from cellulose hydrate (Secon 133, Secon, Göttingen, Federal Republic of Germany), cuprophan (Hemoflow D_2, Fresenius, Oberursel, Federal Republic of Germany), polymethylmethacrylate (Filtryzer, Toray, Tokyo, Japan), ethylene-vinyl alcohol copolymer (KF 101, Salvia, Homburg, Federal Republic of Germany) and polyacrylonitrile (Biospal 2400 S, Hospal, Lyon, France).

Sampling procedures

Whole blood samples were drawn from the patients' arteriovenous fistula prior to dialysis and immediately after completion of dialysis. During the hemodialysis procedure, blood samples were obtained at 30 and 120 min. All blood samples were anticoagulated immediately with sodium citrate.

Assay procedures

Plasma was separated from the sample within 30 min after its collection to prevent leakage of leukocyte constituents. The plasma specimens were stored at −30°C until assayed. The measurement of plasma levels of the granulocyte elastase in complex with α_1-proteinase inhibitor (E-α_1PI) was performed with a highly sensitive enzyme-linked immunoassay [19]. The inhibitory activities of α_1-proteinase inhibitor (α_1PI) and of α_2-macroglobulin (α_2M) were measured with a commercial test systems (Boehringer, Mannheim, Federal Republic of Germany). Plasma concentrations of α_1PI and α_2M were evaluated by nephelometer. Plasma total protein and non-TCA precipitable plasma protein fraction were measured according to the method of Lowry et al [20]. Lysozyme was determined turbidimetrically (Behring, Marburg, Federal Republic of Germany).

Statistics

All values are given as mean ± SEM. For multiple comparisons of data obtained at different time points, an analysis of variance (ANOVA) was used.

Results

The effect of hemodialysis therapy on plasma E-α_1PI levels is shown in Figure 1. Cellulose hydrate membrane caused a maximum E-α_1PI concentration of 1,659.0 ± 256.8 ng/ml ($P < 0.001$). The increase of plasma E-α_1PI value was also significant after 120 min of hemodialysis ($P < 0.01$) in comparison to all other membranes. Cuprophan membrane induced an E-α_1PI increase of 557.5 ± 120.0 ng/ml, whereas maximal E-α_1PI values of 643.0 ± 174.7 ng/ml were measured in the presence of the polymethylmethacrylate membrane. Both membranes caused significantly higher E-α_1PI levels at the end of hemodialysis compared with the polyacrylonitrile membrane (237.8 ± 22.9 ng/ml). Plasma E-α_1PI values were 381.9 ± 54.0 ng/ml at the end of dialysis using the ethylene-vinyl alcohol copolymer membrane, and this increase was only statistically different from the values obtained in the presence of cellulose hydrate membrane.

Plasma α_1PI concentrations and activities are shown in Tables 1 and 2. Both parameters increased slightly during hemodialysis. However, using an analysis of variance

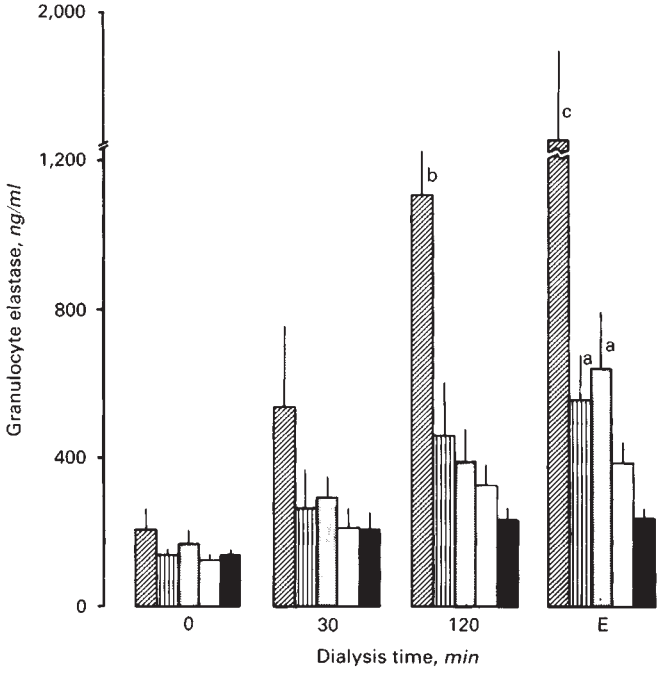

Fig. 1. *Effect of different membrane materials on plasma levels of granulocyte elastase in complex with α_1-proteinase inhibitor (E-α_1PI) 30 and 120 min after the start and at the end (E) of hemodialysis.* Symbols are: ▨, cellulose hydrate; ▥, cuprophan; ⊠, polymethylmethacrylate; □, ethylene-vinyl alcohol copolymer; ■, Polyacrylonitrile. $^aP < 0.05$ in comparison to the polyacrylonitrile membrane at the end (E) of hemodialysis. $^bP < 0.01$ in comparison to all other membranes after 120 min of hemodialysis. $^cP < 0.001$ in comparison to all other membranes at the end (E) of hemodialysis.

(ANOVA) for multiple comparisons of data, the effect of different membrane materials was not significant statistically.

No significant change was found either when comparing plasma α_2M concentrations and activities during hemodialysis using five different dialyzer membranes (Tables 3 and 4).

The effect of different membrane materials on plasma lysozyme concentration is shown in Table 5. In comparison to the cellulose hydrate and cuprophan membranes, there was a significant decrease of plasma lysozyme concentration after 120 min and at the end of hemodialysis using the polymethylmethacrylate membrane. Polyacrylonitrile membrane caused significantly lower plasma lysozyme levels after 30 and 120 min compared with all other membranes and also at the end of hemodialysis, polymethylmethacrylate membrane excepted.

However, plasma concentration of non-TCA precipitable protein decreased during hemodialysis (Fig. 2). The plasma concentrations fell from 886.1 ± 72.9 to 558.5 ± 62.9 μg/ml ($P < 0.001$) in the presence of the cellulose hydrate membrane, from 921.8 ± 59.7 to 556.1 ± 48.7 μg/ml ($P < 0.001$) in the presence of the cuprophan membrane, and from 1,019.4 ± 111.3 to 625.2 ± 70.1 μg/ml ($P < 0.001$) in the presence of the polymethylmethacrylate membrane. The ethylene-vinyl alcohol copolymer dialyzer caused a decrease of non-TCA precipitable protein fraction from 871.9 ± 87.4 to 609.8 ± 68.9 μg/ml ($P < 0.001$) and the polyacrylonitrile dialyzer from 878.1 ± 61.5 to 534.7 ± 75.5 μg/ml ($P < 0.001$; Wilcoxon's paired test). However, using an analysis of variance (ANOVA) for multiple

Table 1. Effect of different membrane materials on plasma concentrations of the α_1-proteinase inhibitor, *mg/dl*

Dialyzer	Before dialysis	30 min	120 min	End of dialysis
Cellulose hydrate (N = 10)	212.5 ± 14.2	221.7 ± 14.1	232.2 ± 17.7	226.5 ± 16.8
Cuprophan (N = 15)	220.8 ± 18.2	233.2 ± 18.5	239.6 ± 16.4	236.0 ± 18.3
Polymethylmethacrylate (N = 10)	236.6 ± 16.0	255.2 ± 18.5	268.6 ± 21.4	268.0 ± 16.8
Ethylene-vinyl alcohol copolymer (N = 15)	222.2 ± 14.2	230.6 ± 14.5	236.2 ± 15.7	232.9 ± 16.6
Polyacrylonitrile (N = 15)	214.1 ± 16.3	222.2 ± 18.3	238.2 ± 22.0	244.4 ± 31.7

Mean values ± SEM.

Table 2. Effect of different membrane materials on plasma activities of the α_1-proteinase inhibitor, *U/ml*

Dialyzer	Before dialysis	30min	120 min	End of dialysis
Cellulose hydrate (N = 10)	1.57 ± 0.20	1.64 ± 0.18	1.65 ± 0.15	1.63 ± 0.15
Cuprophan (N = 15)	1.69 ± 0.11	1.91 ± 0.15	1.94 ± 0.15	1.90 ± 0.14
Polymethylmethacrylate (N = 10)	1.79 ± 0.10	1.95 ± 0.13	2.00 ± 0.12	2.11 ± 0.13
Ethylene-vinyl alcohol copolymer (N = 15)	1.83 ± 0.12	1.86 ± 0.11	1.84 ± 0.12	1.90 ± 0.15
Polyacrylonitrile (N = 15)	1.61 ± 0.11	1.81 ± 0.17	1.86 ± 0.20	1.87 ± 0.23

Mean values ± SEM.

Table 3. Effect of different membrane materials on plasma concentrations of α_2-macroglobulin, *mg/dl*

Dialyzer	Before dialysis	30min	120 min	End of dialysis
Cellulose hydrate (N = 10)	144.4 ± 12.9	142.4 ± 12.1	144.5 ± 11.1	141.7 ± 11.5
Cuprophan (N = 15)	147.6 ± 12.7	147.8 ± 10.3	155.6 ± 10.3	144.4 ± 13.1
Polymethylmethacrylate (N = 10)	142.7 ± 11.0	147.8 ± 13.1	153.2 ± 16.1	153.9 ± 13.3
Ethylene-vinyl alcohol copolymer (N = 15)	160.7 ± 15.3	160.4 ± 16.6	163.0 ± 16.9	166.7 ± 17.1
Polyacrylonitrile (N = 15)	138.2 ± 11.3	144.2 ± 11.2	144.8 ± 10.8	153.2 ± 13.8

Mean values ± SEM.

comparisons of data, the effect of different membrane materials was not significant statistically.

Discussion

In the present study, the effect of different membrane materials (cellulose hydrate, cuprophan, polymethylmethacrylate, ethylene-vinyl alcohol copolymer, and polyacrylonitrile) on the plasma levels of granulocyte elastase during hemodialysis was investigated. Cellulose hydrate membrane caused a maximum level of E-α_1PI of 1,659.0 ± 256.8 ng/ml. The lowest plasma E-α_1PI values were observed using the polyacrylonitrile membrane. Patients dialyzed with polysulfone dialyzers also displayed only very modest increases in their plasma E-α_1PI levels during hemodialysis [21]. Similar results were obtained in patients dialyzed with ethylene-vinyl alcohol copolymer dialyzers. Cuprophan and polymethylmethacrylate membranes caused a significantly higher increase of plasma E-α_1PI values (Fig. 1).

The intensity of complement activation during hemodialysis is determined by the type of dialysis membrane and whether it is new or reused [16, 22–25). Noncellulosic membrane formulations have been reported to be more compatible with blood than the cellulosic membrane [3, 4, 16, 18, 21]. Significant complement activation and anaphylatoxin formation were observed in patients dialyzed with cuprophan hollow-fiber membranes [6–8, 16, 17, 26]. By contrast, hemodialyzers containing polyacrylonitrile membrane promoted very little complement activation [16], a fact that correlates well with our observation that this membrane activates very little PMNs (Fig. 1). However, polymethylmethacrylate surfaces caused only a small degree of complement activation [24], whereas relative high plasma E-α_1PI levels were observed in the present study. The

Table 4. Effect of different membrane materials on plasma activities of α_2-macroglobulin, *U/ml*

Dialyzer	Before dialysis	30min	120 min	End of dialysis
Cellulose hydrate (N = 10)	5.26 ± 0.63	5.77 ± 0.66	5.43 ± 0.53	5.60 ± 0.58
Cuprophan (N = 15)	5.30 ± 0.35	5.71 ± 0.40	5.60 ± 0.38	5.53 ± 0.44
Polymethylmethacrylate (N = 10)	5.74 ± 0.62	5.89 ± 0.63	6.06 ± 0.74	6.15 ± 0.72
Ethylene-vinyl alcohol copolymer (N = 15)	5.14 ± 0.45	5.11 ± 0.49	5.35 ± 0.46	5.39 ± 0.43
Polyacrylonitrile (N = 15)	4.99 ± 0.42	5.15 ± 0.50	5.40 ± 0.41	5.55 ± 0.46

Mean values ± SEM.

Table 5. Effect of different membrane materials on plasma lysozyme concentration, *g/liter*

Dialyzer	Before dialysis	30min	120 min	End of dialysis
Cellulose hydrate (N = 10)	3.53 ± 0.34	3.69 ± 0.32	3.87 ± 0.36	3.84 ± 0.36
Cuprophan (N = 15)	3.87 ± 0.31	3.77 ± 0.35	3.81 ± 0.36	3.80 ± 0.31
Polymethylmethacrylate (N = 10)	3.91 ± 0.31	2.91 ± 0.29	2.35* ± 0.19	2.44* ± 0.23
Ethylene-vinyl alcohol copolymer (N = 15)	3.27 ± 0.15	3.32 ± 0.20	3.33 ± 0.23	3.40 ± 0.24
Polyacrylonitrile (N = 15)	3.37 ± 0.28	1.79** ± 0.17	1.14** ± 0.16	1.66*** ± 0.29

Mean values ± SEM.
* $P < 0.01$ in comparison to the cellulose hydrate and cuprophan membranes.
** $P < 0.01$ in comparison to all other membranes.
*** $P < 0.01$ in comparison to all other membranes except the polymethylmethacrylate.

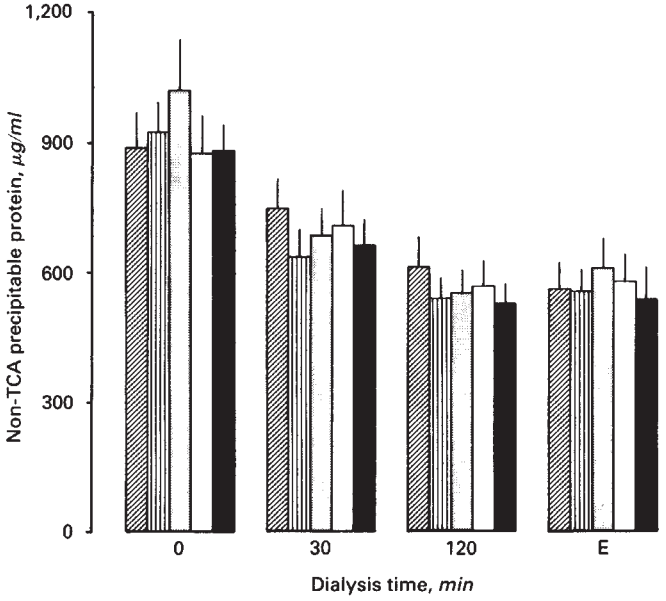

Fig. 2. *Effect of different membrane materials on plasma concentration of non-TCA precipitable protein 30 and 120 min after the start and at the end (E) of hemodialysis.* Symbols are as Fig. 1.

role of different sterilization procedures (gamma radiation: polymethylmethacrylate; ethylene oxide: cellulose hydrate, cuprophan, ethylene-vinyl alcohol copolymer, and polyacrylonitrile) should also be taken into account. Mean plasma E-α_1PI concentrations before hemodialysis were in a range between 127.1 ± 13.6 (ethylene-vinyl alcohol copolymer) and 207.3 ± 58.4 ng/ml (cellulose hydrate). The highest E-α_1PI levels were found during hemodialysis with cellulose hydrate dialyzers. Therefore, the E-α_1PI values measured on the institution of hemodialysis are not the result of the last hemodialysis and not related to the sequence of the use of the different dialyzers. Recent studies from our laboratory showed E-α_1PI concentrations of 112.3 ± 10.3, 151.1 ± 13.2, 174.4 ± 25.0 ng/ml [11] and 188 ± 20 ng/ml [9] before dialysis.

It is evident that daily differences exist in uremic patients. Plasma E-α_1PI levels, however, are always significantly higher than mean E-α_1PI values of healthy controls (70.3 ± 4.4 ng/ml).

It can be argued that results of E-α_1PI, α_1PI, α_2M, and lysozyme could be given not only per ml of plasma but also on a plasma protein basis because large variations in protein concentrations might have been observed in some patients. However, the significance of the changes in plasma E-α_1PI and lysozyme values described was not influenced when calculated on a plasma protein basis.

The control of proteolytic activities in blood and other tissues is exerted, primarily, by nine plasma proteins [27]. Alpha$_1$-proteinase inhibitor (alpha$_1$-antitrypsin) and alpha$_2$-macroglobulin are the major plasma antiproteinases that inactivate granulocyte elastase [27, 28]. Therefore, both the plasma concentrations and the activities of these proteinase inhibitors were determined. There was a significant increase of α_1PI and

α_2M activities (U/ml) and concentrations (mg/dl), but, on a plasma protein basis, these effects were abolished. A previous study [9] also failed to exhibit changes of α_1PI activity and concentration during the first 3 of 5 hr of hemodialysis, when the weight was kept constant. In a further study with 70 patients, a significant increase of plasma α_1PI concentration (mg/dl) was observed during hemodialysis but α_1PI activity remained constant [11].

By contrast, hemodialysis with cuprophan membranes was found to produce a significant decrease of alpha$_1$-antitrypsin. This decrease was particularly obvious during the first 30 min, but it lasted for 240 min in most patients [29]. It is quite possible that the liberation from granulocyte elastase together with other lysosomal enzymes might lead to repeated local proteinase-antiproteinase imbalances. It was suggested that proteases released from granulocytes are rapidly bound to alpha$_1$-antitrypsin, causing a decrease of this antiprotease [29]. Our results do not, however, support this theory.

Specific granules of PMNs contain lysozyme [30, 31], collagenase [32], B-12 binding protein, and lactoferrin. Azurophil granules also contain lysozyme [30, 31]. Leukocyte activation of cell death is associated with the release of lysozyme [33] and lactoferrin [18, 34]. It was shown recently that leukocytes leaving the cellophan dialyzer had a significantly decreased lysozyme content [33]. In the present study, the plasma lysozyme levels before, during, and after hemodialysis were measured. Before hemodialysis, plasma lysozyme values were significantly elevated (Fig. 1.) compared with healthy controls (0.3 to 0.9 g/liter). Patients dialyzed with cellulose hydrate, cuprophan, or ethylene-vinyl alcohol copolymer dialyzers failed to exhibit significant changes of plasma lysozyme values. By contrast, there was a significant decrease of plasma lysozyme concentration during and immediately after hemodialysis in the presence of polyacrylonitrile membrane. Lysozyme is a basic polypeptide composed of 120 amino acids and with a molecular weight of 15,000. The mechamism of lowered plasma lysozyme concentration during hemodialysis with polyacrylonitrile or polymethylmethacrylate membranes is unclear. We cannot exclude that this basic polypeptide binds on these two dialyzers. It is unlikely that lysozyme is eliminated during hemodialysis, since plasma levels remain constant using the ethylene-vinyl alcohol copolymer hollow fiber, which is superior in the permeability of middle molecular weight substances and proteins compared with the other cellulosic and noncellulosic membranes.

Trichloroacetic acid (TCA) is widely used as a protein coagulant in the preparation of protein-free sera. The elimination of bound water and its replacement by the strongly non-polar Cl_3 C.C-radical destroys the solubility of proteins [35]. The mean values of this small molecular protein fraction obtained from patients with posttraumatic acute renal failure were 1.65 ± 0.11 mg/ml [36], compared with 1.036 ± 0.029 mg/ml from patients on regular hemodialysis treatment [11] and 0.246 ± 0.019 mg/ml from health controls [36]. Non-TCA-precipitable plasma protein fraction decreased during hemodialysis, independent of the dialyzer used (Fig. 2.).

In summary, the observations reported here are quite compatible with what is currently known about the biocompatibility of dialysis membranes. Polyacrylonitrile dialyzers provoke minimal C3a [16, 22] and E-α_1PI (Fig. 1.)formation and consistently fail to induce extensive leukopenia [4, 21, 22]. The results of the comparison of the effects of five different dialyzers on the plasma content in elastase complexed with α_1-proteinase inhibitor (E-α_1PI) indicate that this parameter may be a further index of biocompatibility.

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