N. Friedel, R. Hetzer, D. Royston (Eds.)

Blood Use in Cardiac Surgery

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The Editors:
Dr. N. Friedel
Prof. Dr. R. Hetzer
Deutsches Herzzentrum Berlin
Augustenburger Platz 1
1000 Berlin 65, FRG

Dr. D. Royston
Harefield Hospital
Hill End Road
Harefield
Middlesex
UB9 6JH

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Investigation on the Mechanisms of Action of Aprotinin in Cardiac Surgery

W. Dietrich[1], M. Spannagl[2], M. Jochum[3], P. Wendt[4], A. Barankay[1], J. A. Richter[5]

[1] Staff Anesthesiologist, Institute for Anesthesiology, German Heart Center, Munich, [2] Research Fellow, Internal Medicine, University Clinic Munich, [3] Research Fellow, Department of Surgery, Division of Clinical Chemistry, University Clinic Munich, [4] Research Fellow, Department of Experimental Surgery, Technical University Munich, [5] Chairman, Institute for Anesthesiology, German Heart Center Munich

Introduction

Blood loss and bleeding tendency with the consequence of homologous blood transfusion still present a major problem in cardiac surgery. Many efforts have been made to influence the bleeding tendency in open-heart surgery by pharmacological means. Aprotinin is a naturally occurring enzyme inhibitor derived from bovine lungs. It acts on trypsin, plasmin, tissue-kallikrein and, to lesser degree, on plasma-kallikrein [1, 2]. Moreover, it is reported to have direct platelet-preserving properties in very high dosages [3]. Aprotinin has been used in Europe for a long time in varying indications, but only since the results published by Royston et al. [4] who applied very high dosages of aprotinin, has this drug been regarded with increased interest. Recently, these results were corroborated by several studies [5—9].

Based upon previous investigations [7], we postulated that the clinical effect of aprotinin is mainly due to the inhibition of the contact phase of coagulation. During CPB this system is activated by contact of blood with artificial surfaces of the extracorporeal circuit [10]. However, the postoperative bleeding tendency after cardiac surgery seems to be primarily due to impaired platelet function [11]. But the mechanism underlying the benefit from aprotinin has not yet been elucidated completely.

The aim of our present prospective, double-blind, placebo controlled study was to obtain further information about the mode of action of aprotinin.

Methods

Forty patients scheduled for elective primary myocardial revascularization gave informed consent to participate in this study, which had been approved by the local ethics committee. The study group comprised only male patients with preoperative normal left ventricular function (EF $>$ 40%, LVEDP $<$ 20 mmHg), with a preoperative hemoglobin concentration $>$ 13.5 g/dl, and who were not receiving preoperative anticoagulant treatment or antiplatelet medication.

Patients were randomly assigned to one of two groups: the aprotinin group (group A) and the control (group C). The following dosage regime of aprotinin was applied:

after induction of anesthesia and prior to surgery, patients received a loading dose of 2×10^6 KIU aprotinin over a 15-min period, followed by a continuous infusion of 5×10^5 KIU per hour administered by an infusion pump during the whole time of surgery. An additional bolus of 2×10^6 KIU was added to the pump prime of the heart-lung machine. Patients of group C received an equal volume of saline.

The indication for intra- and postoperative transfusion of homologous blood or blood products was defined in the study protocol as a hematocrit of less than 30 %.

Anesthetic, operative, and bypass management were standardized. Patients were heparinized with 125 units/kg mucosa heparin. Further heparin (125 units/kg) was administered if the activated clotting time (ACT) decreased below 400 s. The extracorporeal circuit consisted of a bubble oxygenator, which was primed with 1400 ml crystalloid solution. After completion of CPB, residual heparin was neutralized with protamine chloride in a ratio of 1.5 mg per 125 units of the initial heparin dosage.

Blood transfusions needed until discharge from the hospital were recorded. Intraoperative blood loss was assessed by weighing the gauzes and sponges and measuring the content of the suction reservoir. Postoperative blood loss was measured as cumulative chest tube output 6, 12, and 24 h postoperatively, as well as at the removal of the chest tubes.

Blood samples were taken at the following times: 1) after induction of anesthesia and prior to aprotinin infusion; 2) prior to heparin administration; 3) 5 min and 4) 30 min after start of CPB; 5) at the end of CPB; 6) after chest closure. After discarding the first 10 ml, blood was drawn into EDTA tubes or into ACC solution (4 : 1).

Tissue plasminogen activator (tPA) concentration, the split products of cross-linked fibrin (D-dimers), total degradation products of fibrinogen and fibrin (FSP), and the complex of thrombin with antithrombin III (TAT) [12] were determined by sandwich ELISA's using polyclonal as well as monoclonal antibodies. Results are given in ng/ml. Aprotinin plasma concentrations were quantified by means of a competitive ELISA, according to Müller-Esterl et al. [13]. Spontaneous fibrinolytic activation in the native samples, as well as in their euglobulin fraction, was estimated on plasminogen containing human fibrin plates [14].

To determine the ACT, the Hemochron 800 (International Technidine Corp., New Jersey, USA) was used. ACT measurements were performed every 30 min and at all intraoperative measurement times.

Summary data of all variables are expressed as means $\pm$ standard deviation (SD). Analysis of variance (ANOVA) was used if appropriate. Chi-square test was applied for categorical data. A p value less than 0.05 was considered statistically significant.

Results

Patients' demographic data were comparable in terms of age, weight, operation, and CPB time. Thirteen patients in group C and 11 patients in group A had an additional internal mammary artery implantation.

The assessed intraoperative blood loss was 636 ± 322 ml in group C and 363 ± 159 ml in group A ($p < 0.05$). The mean cumulated loss 6, 12 and 24 h postoperatively was 721 ± 471 ml, 894 ± 491 ml, and 1169 ± 605 ml in group C, and 303 ± 209 ml, 399 ± 251 ml, and 584 ± 295 ml in group A. The total postoperative blood loss until removal of the chest tubes was 1431 ± 760 ml in group

C, and 738 ± 411 ml in group A ($p < 0.05$) (Fig. 1). The mean amount of intra- and postoperatively transfused homologous blood was 838 ± 963 ml/patient in group C and 163 ± 308 ml in group A, respectively ($p < 0.05$). 2.3 ± 2.2 units of homologous blood or blood products were given in group C and 0.63 ± 0.96 units/patient in group A ($p < 0.05$). Twenty-five % of the patients in group C and 63% in group A were discharged from the hospital without receiving any banked blood or homologous blood products. The intraoperative blood loss correlated significantly with CPB time in group C, whereas it did not in group A.

The ACT, which was in a comparable range preoperatively in the two groups, was significantly increased 5 min prior to administration of heparin in group A (141 ± 13 vs 122 ± 25 s) and remained significantly higher until antagonizing the effect of heparin after CPB. The aPTT was also significantly increased before heparin in group A (34 ± 2.8 vs 74 ± 7.3 s) ($p < 0.05$) and remained significantly prolonged until 2 h post surgery (66 ± 23 vs 45 ± 25 s).

The concentration of the TAT complex 30 min after the start of CPB and at the end of CPB showed significant differences ($p < 0.05$) with 48 ± 21 and 82 ± 42 ng/ml (group C) compared to 24 ± 11 and 42 ± 14 ng/ml in group A.

The concentration of the split products of the cross-linked fibrin (D-dimers) increased in both groups during surgery. However, the increase was less in the aprotinin group and was 532 ± 1425 and 497 ± 1398 ng/ml, 30 m in after onset of CPB and at the end of CPB, respectively, significantly less than the values of group C (2155 ± 2300 and 3131 ± 2755 ng/ml). The FSP concentrations showed a similar course (Fig. 2). At the end of CPB the concentration was 10 824 ± 7261 ng/ml in group C and 2510 ± 3932 ng/ml in the aprotinin group ($p < 0.05$).

The fibrin plates revealed increased fibrinolytic activity during the entire course of CPB in the control group. In the native samples fibrinolytic activity was evident

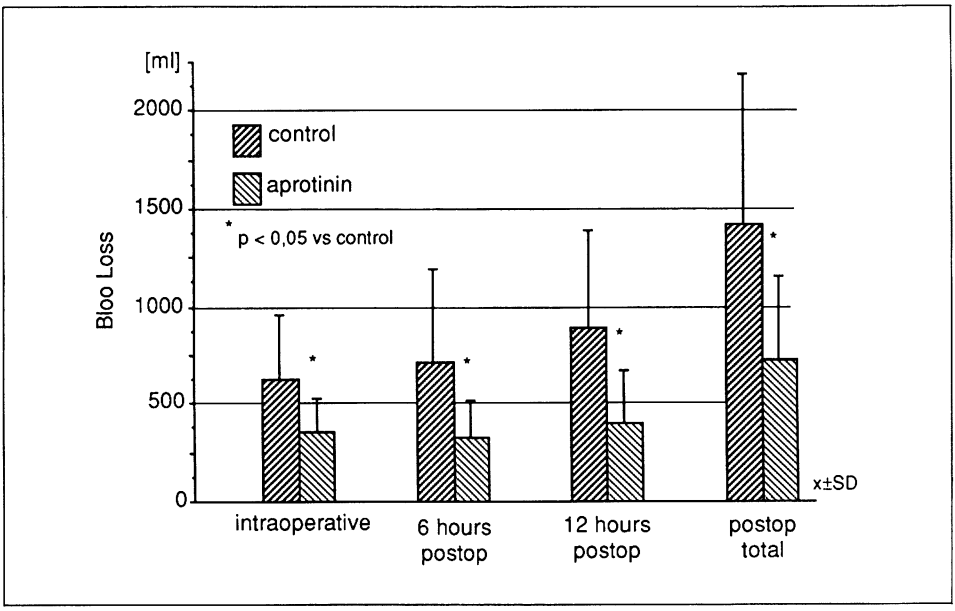

Fig. 1. Intra- and postoperative blood loss. The postoperative data are cumulative values.

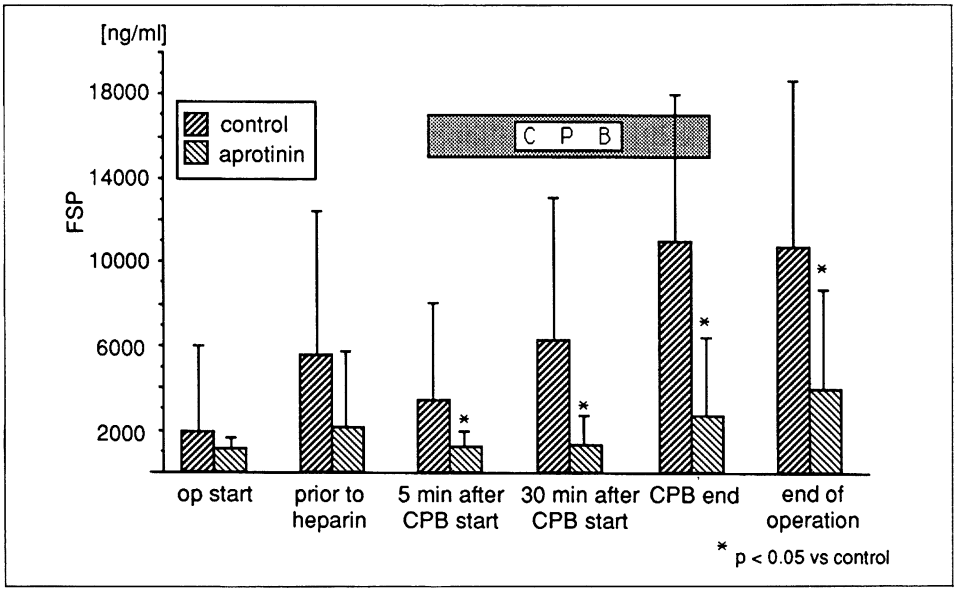

Fig. 2. Total degradation products of fibrinogen and fibrin. Values shown in the graph are mean ± SD. There was a significant increase during the whole period of CPB in the control group compared to the aprotinin group.

in 12 patients/5 min and in 13 patients/30 min after onset of CPB in the control group, whereas this could not be demonstrated 5 min after start of CPB, and in 8 patients/30 min after start of CPB in the aprotinin group ($p < 0.05$). The tPA concentration was not significantly different during the course of surgery.

The aprotinin plasma concentration (Fig. 3) demonstrated an increase from 152 ± 61 KIU/ml prior to heparin to 335 ± 106 KIU/ml 5 min after onset of CPB. Thereafter, a continuous decrease was found until the end of CPB (191 ± 62 KIU/ml).

The postoperative course of all patients was uneventful. There were no clinically relevant side effects that could be attributed to aprotinin treatment.

Discussion

The present study demonstrates the influence of high-dose aprotinin treatment on intra- and postoperative blood loss. Comparing homogeneous patient groups, a significant reduction of intra- and postoperative blood loss was found in the aprotinin-treated group. This reduction led to a concomitant saving of homologous blood. The postoperative blood loss was reduced by 48%, whereas the banked blood requirement was diminished by nearly 80%.

The differences between the two groups with regard to bleeding and banked blood requirement agree with the results of other authors applying the same or a similar aprotinin dosage regime. The blood-saving effect in these studies varied between 43% [7] and 88% [4] (Table 1). All studies using aprotinin during coronary artery bypass grafting showed nearly identical reductions in blood loss.

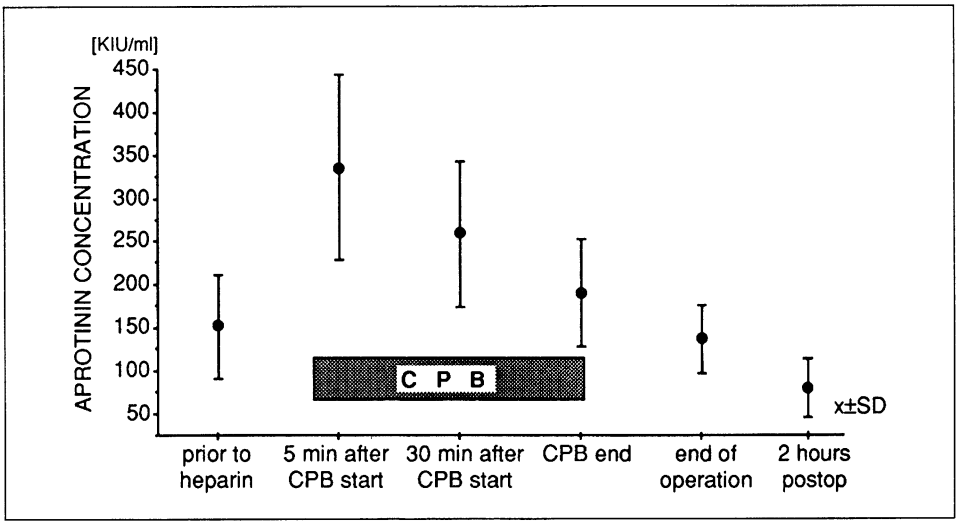

Fig. 3. Aprotinin plasma level. The peak value 5 min after the onset of CPB represents the bolus of 2×10^6 KIU aprotinin given to the pump prime. Despite the continuous infusion of 5×10^5 KIU aprotinin/h the concentration decreased towards the end of CPB and towards the end of operation.

Table 1. Studies on aprotinin in open-heart surgery.

Autor	Year	Type of operation	No. of patients	Blood loss	Blood saving
Royston et al. [4]	1987	Redos	22	−80%	88%
v. Oeveren et al. [15]	1987	CABG	22	−47%	−50%
Bidstrup et al. [6]	1989	CABG	80	−46%	−83%
Dietrich et al. [7]	1989	Miscellaneous	152	−29%	−43%
Fraedrich et al. [8]	1989	CABG	80	−46%	−43%
Henze et al. [16]	1989	Miscellaneous	380	−35%	−40%
Dietrich et al. [17]	1989	CABG	40	−48%	−80%
Dietrich et al. [18]	1990	Miscellaneous	1784	−34%	−53%

Nevertheless, the mode of action of aprotinin is not yet completely clear. Capillary bleeding and oozing in cardiac surgery are supposed to be due to impaired platelet function [11, 19]. The most important consequence of CPB is the loss of platelet aggregability [10]. The influence of aprotinin on platelet adhesive receptors could be demonstrated in one study [9]. Consequently, a direct platelet-preserving property of aprotinin has been postulated [9, 15].

The surface-mediated activation of the contact system of coagulation involves the interaction of Factor XII (Hageman factor) and kallikrein (besides high molecular weight kininogen and Factor XI) [20]. Aprotinin may inhibit kallikrein. Without the amplifying effect of kallikrein on the conversion of Factor XII to XIIa the contact phase activation is inhibited or takes place only slowly. Major consequences of the surface-mediated activation are the stimulation of both the intrinsic pathway of coagulation [21] with the effect of thrombin formation and the propagation of the fibrinolytic pathway leading to plasminogen activation. Thrombin is a powerful

platelet activator. Therefore, it is conceivable that the effect of aprotinin on platelets is secondary to the inhibition of the contact system of coagulation (Fig. 4)

Aprotinin is a strong plasmin inhibitor [22]. While we observed differences in the results of the fibrin plates between the groups, we could no detect any significant difference in the course of tPA concentration. The current findings indicate, that endothelial activation of fibrinolysis is not inhibited by aprotinin, whereas the activation via plasma kallikrein is attenuated. The course of the ACT, which is an indicator of contact activation [23], as well as the aPTT elevation with aprotinin treatment refer to the inhibition of the contact phase of coagulation. These hemostasiological results strongly suggest, that the inhibition of the contact phase of coagulation is the primary effect of aprotinin and is thus responsible for its blood-saving effect. This finding is in contrast to the interpretation given by van Oeveren et al. [9, 15], who focused on the direct platelet protective effect of aprotinin on the specific platelet receptors.

The rationale for choosing the given aprotinin dose regime was to get a constant aprotinin plasma level of more than 200 KIU/ml, which is supposed to be the threshold of plasma kallikrein inhibition [24]. The course of plasma aprotinin level revealed that aprotinin dosage was not able to maintain a stable concentration throughout CPB. However, the concentration exceeded 200 KIU/ml after onset of CPB. The course of the fibrin-fibrinogen split products and the results of the fibrin plates demonstrated that aprotinin was not able to suppress the ongoing hemostatic activation totally. All parameters showed an increase towards the end of CPB. Further investigation is needed to ascertain whether this tendency can be prevented with an even higher dose regime of aprotinin during CPB.

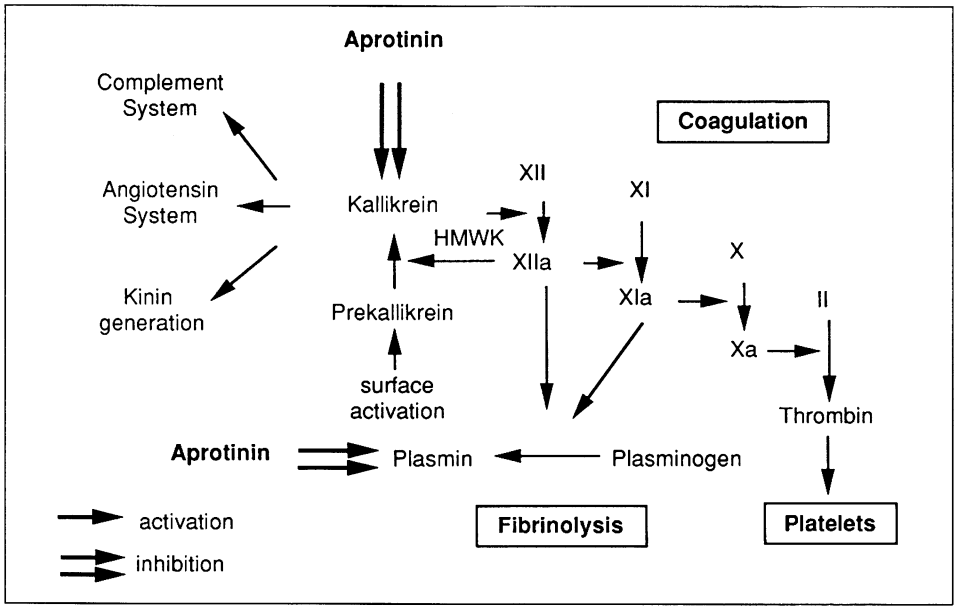

Fig. 4. Aprotinin action on the hemostatic system.

The results of this study suggest that the most likely mechanism of action of aprotinin is the inhibition of the contact phase of coagulation. This is due to the ability of aprotinin to inhibit kallikrein. It acts supplementary to heparin on coagulation. The combination of both drugs provides an enhanced anticoagulatory effect. However, because aprotinin only acts on the contact phase of coagulation, it is not recommended to reduce the heparin dosage under aprotinin. The better preserved platelet function and the inhibition of fibrinolysis is secondary to the better preserved coagulation system.

In conclusion, high-dose aprotinin treatment has a highly beneficial effect on the hemostatic mechanism during and after CPB, leading to a substantial reduction of intra- and postoperative bleeding tendency. The most likely mechanism of aprotinin action is the inhibition of kallikrein and not a direct platelet-preserving property.

References

1. Emerson FE (1989) Pharmacology of aprotinin and efficacy during cardiopulmonary bypass. Cardiovasc Drug Rev 7: 127—140
2. Fritz H (1985) The target enzymes of aprotinin in vitro and in vivo. Proteolyse und Proteinaseinhibition in der Herz- und Gefäßchirurgie, 143—154
3. Aoki N, Naito K, Yoshida N (1978) Inhibition of platelet aggregation by protease inhibitors. Possible involvement of proteases in platelet aggregation. Blood 52: 1—12
4. Royston D, Taylor KM, Bidstrup BP, Sapsford RN (1987) Effect of aprotinin on need for blood transfusion after repeat open-heart surgery. Lancet, ii: 1289—1291
5. Alajmo F, Calamai G, Perna AM et al (1989) High-Dose Aprotinin: Hemostatic effects in open-heart operations. Ann Thorac Surg 48: 536—539
6. Bidstrup BP, Royston D, Sapsfort RN, Taylor KM (1989) Reduction in blood loss and blood use after cardiopulmonary bypass with high dose aprotinin (Trasylol). J Thorac Cardiovasc Surg 97: 364—372
7. Dietrich W, Barankay A, Dilthey G, et al (1989) Reduction of homologous blood requirement in cardiac surgery by intraoperative aprotinin application — clinical experience in 152 cardiac surgical patients. Thorac Cardiovasc Surgeon 37: 92—98
8. Fraedrich G, Weber C, Bernard C, Hettwer A, Schlosser V (1989) Reduction of blood transfusion requirement in open-heart surgery by administration of high doses of aprotinin — preliminary results. Thorac Cardiovasc Surgeon 37: 89—91
9. van Oeveren W, Harder MP, Roozendaal KJ, Eijsman L, Wildevuur CRH (1990) Aprotinin protects platelets against the initial effect of cardiopulmonary bypass. J Thorac Cardiovasc Surg 99: 788—797
10. Edmunds LH, Ellison N, Colman WR, et al (1982) Platelet function during cardiac operation — comparison of membran and bubble oxygenators. J Thorac Cardiovasc Surg 83: 805—812
11. Harker LA (1986) Bleeding after cardiopulmonary bypass. N Engl J Med 314: 446—448
12. Bauer KA, Rosenberger RD (1984) Thrombin generation in acute promyelocytic leukemia. Blood 64: 64
13. Müller-Esterl W, Oettl A, Trucheit E, Fritz H (1984) Monitoring of aprotinin plasma levels by an enzyme-linked immunosorbent assay (ELISA). Fresenius Z Anal Chem 317: 718
14. Wendt P, Fritsch A, Schulz F, Wunderlich G, Blümel G (1984) Proteinases and inhibitors in plasma and peritoneal exudate in acute pankreatitis. Hepato-gastroenterol 31: 277—281
15. van Oeveren W, Jansen NJG, Bidstrup BP, et al (1987) Effects of aprotinin on hemostatic mechanisms during cardiopulmonary bypass. Ann Thorac Surg 44: 640—645
16. Henze R, Dietrich W, Hähnel C, Sebening F, Richter JA (1989) Verminderter Blutverbrauch in der Herzchirurgie durch intraoperative Aprotininapplikation. Anästhesist 38: 510
17. Dietrich W, Spannagl M, Jochum M, Wendt P, Sebening F, Richter JA (1989) Reduction of homologous blood requirement in cardiac surgery using high-dose aprotinin. Anesthesiology (Suppl)

18. Dietrich W, Hähnel C, Richter JA (1990) Routine application of high-dose aprotinin in open-heart surgery — a study on 1784 patients. Anesthesiology (Suppl), in press
19. Edmunds LH (1989) Letter to the editor: Blood platelets and bypass. J Thorac Cardiovasc Surg 97: 470—477
20. Colman RW (1984) Surface-mediated defense reactions. The plasma contact activation system. J Clin Invest 73: 1249—1253
21. Carvalho AC, De Marinis S, Scott CF, Silver LD, Schmaier AH, Colman RW (1988) Activation of the contact system of plasma proteolysis in the adult respiratory distress syndrome. J Lab Clin Med 112: 270—277
22. Verstraete M (1985) Clinical application of inhibitors of fibrinolysis. Drugs 29: 236—261
23. Hattersley PG (1966) Activated coagulation time of whole blood. JAMA 196: 436—440
24. Fritz H, Wunderer G, (1983) Biochemistry and applications of aprotinin, the kallikrein inhibitor from bovine organs. Arzneim Forsch/Drug Res 33: 479—494

Authors' address:
W. Dietrich, M.D.
Institute for Anesthesiology
German Heart Center Munich
Lothstraße 11
8000 Munich 2, FRG