

# **PROTEASES II**

## **Potential Role in Health and Disease**

Edited by

**Walter H. Hörl**

University of Freiburg  
Freiburg, Federal Republic of Germany

and

**August Heidland**

University of Würzburg  
Würzburg, Federal Republic of Germany

**PLENUM PRESS • NEW YORK AND LONDON**

---

Library of Congress Cataloging in Publication Data

International Symposium on Proteases: Potential Role in Health and Disease (2nd: 1987: Rothenburg ob der Tauber, Germany)

Proteases II: potential role in health and disease.

(Advances in experimental medicine and biology; v. 240)

"Proceedings of the Second International Symposium on Proteases: Potential Role in Health and Disease, held May 17-20, 1987 in Rothenburg ob der Tauber, Federal Republic of Germany"—T.p. verso.

Includes bibliographies and index.

1. Proteolytic enzymes—Congresses. 2. Proteolytic enzymes—Pathophysiology—Congresses. 3. Proteolytic enzyme inhibitors—Congresses. I. Hörl, Walter H. II. Heidland, August. III. Title. IV. Series. [DNLM: 1. Peptide Hydrolases—congresses. 2. Protease Inhibitors—congresses. W1 AD559 v.240 / QU 136 I61 1987]

QP609.P78I57 1987

612'.01516

88-25549

ISBN 0-306-43018-5

---



Proceedings of the Second International Symposium on Proteases:  
Potential Role in Health and Disease, held May 17-20, 1987, in  
Rothenburg ob der Tauber, Federal Republic of Germany

© 1988 Plenum Press, New York  
A Division of Plenum Publishing Corporation  
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

## CONTENTS

### I PHYSIOLOGY AND PATHOPHYSIOLOGY OF PROTEASES AND THEIR INHIBITORS

|   |    |
|---|----|
| Aspartic Proteinases and Inhibitors for their Control in Health and Disease .....                         | 1  |
| J. Kay, R. A. Jupp, C. G. Norey, A. D. Richards, W. A. Reid, R. T. Taggart, I. M. Samloff, and B. M. Dunn |    |
| Human Neutral Endopeptidase 24.11 (NEP, Enkephalinase); Function, Distribution and Release .....          | 13 |
| E. G. Erdös and R. A. Skidgel   |    |
| Neutrophil Elastase and Cathepsin G: Structure, Function and Biological Control .....                     | 23 |
| W. Watorek, J. Farley, G. Salvesen, and J. Travis   |    |
| The Degradation of Collagen by a Metalloproteinase from Human Leucocytes .....                            | 33 |
| U. Kohnert, R. Oberhoff, J. Fedrowitz, U. Bergmann, J. Rauterberg, and H. Tschesche                       |    |
| Plasma Membrane Proteinases as Useful Tool in Histochemical Toxicology .....                              | 45 |
| R. Graf and R. Gossrau  |    |
| Activation of Leukocytes During Prolonged Physical Exercise .....   | 57 |
| K. Kokot, R. M. Schaefer, M. Teschner, U. Gilge, R. Plass, and A. Heidland                                |    |
| Inhibition of Human Neutrophil Elastase by Polyguanylic Acid and other Synthetic Polynucleotides .....    | 65 |
| S. Simon, M. Vered, A. Rinehart, J. Cheronis, and A. Janoff   |    |
| Inhibition of Human Neutrophil Elastase by Acid-Soluble Inter-Alpha-Trypsin Inhibitor .....               | 75 |
| A. Gast and J. G. Bieth   |    |
| Development of Eglin c as a Drug: Pharmacokinetics .....  | 83 |
| H. P. Nick, A. Probst, and H. P. Schnebli   |    |

|  |     |
|--|-----|
| Monoclonal Antibodies Recognizing Inter-Alpha-<br>Trypsin-Inhibitor and its Related Fragments -<br>Evidence for the Involvement of the<br>Proteinase Inhibitor in Cutaneous (Patho-)<br>Physiology ..... | 89  |
| C. Justus, K. Hochstrasser, and M. D. Kramer   |     |
| Inhibition of Human Chymotrypsin-Like Proteases by<br>Alpha-1-Proteinase Inhibitor and Alpha-1-<br>Antichymotrypsin .....  | 97  |
| A. Hayem, D. Marko, A. Laine, and M. Davril  |     |
| Immunoreactive Pancreatic Secretory Trypsin<br>Inhibitor in Gastrointestinal Mucosa .....  | 101 |
| M. Bohe, C. Lindström, and K. Ohlsson  |     |

## II PROTEASES AND LUNG

|  |     |
|--|-----|
| Semisynthetic Inhibitors of Human Leukocyte Elastase<br>and their Protective Effect on Lung Elastin<br>Degradation in vitro .....                                      | 107 |
| J. Beckmann, A. Mehlich, H. R. Wenzel, and<br>H. Tschesche   |     |
| Human Bronchial Proteinase Inhibitor:Rapid<br>Purification Procedure and Inhibition of<br>Leucocyte Elastase in Presence and in Absence<br>of Human Lung Elastin ..... | 115 |
| C. Boudier, D. Carvallo, M. Bruch, C. Roitsch,<br>M. Courtney, and J. G. Bieth   |     |
| Functional Studies of Human Secretory Leukocyte<br>Protease Inhibitor .....  | 123 |
| K. Ohlsson, M. Bergenfeldt, and P. Björk   |     |
| The Role of Chymase in Ionophore-Induced Histamine<br>Release from Human Pulmonary Mast Cells .....  | 133 |
| T. Hultsch, M. Ennis, and H. H. Heidtmann  |     |
| Proteolytic Activities in Bronchoalveolar Lavage<br>Fluid Correlate to Stage and Course of<br>Interstitial Lung Disease .....  | 137 |
| M. Schmidt and E. Brugger  |     |
| Behaviour of Angiotensin Converting Enzyme,<br>Hydroxyproline and some Protease Inhibitors<br>in Pulmonary Sarcoidosis .....   | 145 |
| M. Masiak, B. Podwysocki, and A. Gajewska  |     |
| Experimental Studies on the Adult Respiratory<br>Distress Syndrome: Elastase Infusion in<br>Normal and Agranulocytic Minipigs .....                                    | 149 |
| H. Burchardi and T. Stokke   |     |

### III PROTEASES AND LIVER

|  |     |
|--|-----|
| Arginylation, Surface Hydrophobicity and Degradation<br>of Cytosol Proteins from Rat Hepatocytes .....   | 159 |
| P. Bohley, J. Kopitz, and G. Adam  |     |
| Proteinase Inhibitors as Acute Phase Reactants:<br>Regulation of Synthesis and Turnover .....  | 171 |
| A. Koj, D. Magielska-Zero, A. Kurdowska,<br>and J. Bereta  |     |
| Regulation of Proteinase Activity by High Molecular<br>Weight Inhibitors: Biosynthesis of Rat Alpha-<br>Macroglobulins .....   | 183 |
| T. Geiger, T. Andus, D. Kunz, M. Heisig,<br>J. Bauer, N. Northoff, F. Gauthier,<br>T.-A. Tran-Thi, K. Decker, and P. C. Heinrich                                       |     |
| Induction of the Proteinase Inhibitor Alpha-2-<br>Macroglobulin in Rat Hepatocytes by a<br>Monocyte-Derived Factor .....   | 191 |
| T. Andus, H. Northoff, J. Bauer, U. Ganter,<br>D. Männel, T.-A. Tran-Thi, K. Decker, and<br>P. C. Heinrich   |     |
| Astrocytes Synthesize and Secrete Alpha-2-Macro-<br>globulin: Differences Between the Regulation<br>of Alpha-2-Macroglobulin Synthesis in Rat<br>Liver and Brain ..... | 199 |
| J. Bauer, P.-J. Gebicke-Haerter, U. Ganter,<br>I. Richter, and W. Gerok  |     |
| Characterization of Different Forms of Dipeptidyl<br>Peptidase IV from Rat Liver and Hepatoma by<br>Monoclonal Antibodies .....  | 207 |
| S. Hartel, C. Hanski, R. Neumeier, R. Gosrau,<br>and W. Reutter  |     |

### IV MUSCLE PROTEIN DEGRADATION

|  |     |
|--|-----|
| Non-Lysosomal, High-Molecular-Mass Cysteine<br>Proteinases from Rat Skeletal Muscle .....            | 215 |
| B. Dahlmann, L. Kuehn, F. Kopp, H. Reinauer,<br>and W. T. Stauber                                    |     |
| Role of Factors Derived from Activated Macrophages<br>in Regulation of Muscle Protein Turnover ..... | 225 |
| V. E. Baracos  |     |
| Responses of Lysosomal and Non-Lysosomal Proteases<br>to Unloading of the Soleus .....               | 235 |
| E. J. Henriksen, S. Satarug, M. E. Tischler,<br>and P. Fürst   |     |

|  |     |
|--|-----|
| Cathepsin B and D Activity in Human Skeletal<br>Muscle in Disease States .....   | 243 |
| G. Guarnieri, G. Toigo, R. Situlin,<br>M. A. Del Bianco, and L. Crapesi  |     |
| Hormonal Regulation of Muscle Protein Catabolism<br>in Acutely Uremic Rats: Effect of Adrenalectomy<br>and Parathyroidectomy ..... | 257 |
| R. M. Schaefer, M. Moser, P. Kulzer, G. Peter,<br>A. Heidland, W. H. Hörl, and S. G. Massry  |     |

## V PROTEASES, KIDNEY AND UREMIA

|   |     |
|---|-----|
| Relation Between Urinary Proteinases and Proteinuria<br>in Rats with a Glomerular Disease .....                                   | 267 |
| J.-C. Davin, M. Davies, J.-M. Foidart,<br>J. B. Foidart, C. A. Dechenne, and P. R. Mahieu   |     |
| Characterization and Clinical Role of Glomerular<br>and Tubular Proteases from Human Kidney .....                                 | 275 |
| J. E. Scherberich, G. Wolf, C. Stuckhardt,<br>P. Kugler, and W. Schoeppe  |     |
| Effect of Glomerular Proteinuria on the Activities<br>of Lysosomal Proteases in Isolated Segments<br>of Rat Proximal Tubule ..... | 283 |
| C. J. Olbricht  |     |
| Meprin Phenotype and Cyclosporin A Toxicity in<br>Mice .....  | 293 |
| J. F. Reckelhoff, S. S. Craig, R. J. Beynon,<br>and J. S. Bond  |     |
| Potential Role of Lysosomal Proteases in Gentamicin<br>Nephrotoxicity .....   | 305 |
| C. J. Olbricht, E. Gutjahr, M. Fink, and<br>K. M. Koch  |     |
| Urinary Proteinase Activity in Patients with<br>Acute Renal Failure after Trauma and Kidney<br>Transplantation .....              | 309 |
| C. Wanner, S. Greiber, G. Kirste, P. Schollmeyer,<br>and W. H. Hörl   |     |
| Mechanisms for Activation of Proteolysis in<br>Uremia .....   | 315 |
| W. E. Mitch   |     |
| Evidence for the Role of Proteinases in Uremic<br>Catabolism .....  | 323 |
| R. M. Schaefer, M. Teschner, G. Peter,<br>J. Leibold, P. Kulzer, and A. Heidland  |     |
| Eglin C Fails to Reduce Catabolism in Actuely Uremic<br>Rats .....  | 331 |
| M. Teschner, R. M. Schaefer, C. Rudolf,<br>P. Kulzer, G. Peter, and A. Heidland   |     |

|  |     |
|--|-----|
| Evidence for Protein Split Products in Plasma<br>of Patients with Acute Renal Failure .....  | 339 |
| M. Haag, H. E. Meyer, P. Schollmeyer, and<br>W. H. Hörl  |     |
| Proteases and Antiproteases at Different Vascular<br>Sites in Renal Failure .....  | 345 |
| K. Bausewein, K. Schafferhans, R. Götz,<br>U. Gilge, E. Heidbreder, and A. Heidland  |     |
| Protease Histochemistry in Normal and Uremic<br>Rats .....   | 351 |
| R. Gossrau, A. Heidland, and J. Haunschild   |     |
| Total Kininogen Levels, Plasma Renin Activity,<br>Dopamine-Beta-Hydroxylase and Plasma<br>Catecholamines in Chronic and Acute<br>Renal Failure ..... | 361 |
| K. Marczewski, A. Ksiazek, J. Solski, and<br>Z. Pachucki   |     |

## VI PROTEOLYTIC ENZYMES DURING EXTRACORPORAL CIRCULATION

|   |     |
|---|-----|
| Biocompatibility of Dialysis Membranes: Factor H<br>Binding Correlates Inversely with Complement<br>Activation Indicating a Local Imbalance of<br>Involved Proteases/Anti-Proteases ..... | 365 |
| E. W. Rauterberg and E. Ritz  |     |
| Hemodialysis with Curophane Membranes Leads to<br>Alteration of Granulocyte Oxidative Metabolism<br>and Leukocyte Sequestration in the Lung .....   | 377 |
| G. Kolb, H. Schönemann, W. Fischer, K. Bittner,<br>H. Lange, H. Höffken, V. Damann, K. Joseph,<br>and K. Havemann   |     |
| Effect of Immunosuppression on the Release of Main<br>Granulocyte Components: In Vivo and in Vitro<br>Studies .....   | 385 |
| C. Wanner, B. Simon, A. Gösele, W. Riegel,<br>P. Schollmeyer, and W. H. Hörl  |     |
| Release of Granulocyte Proteins During Cardiopulmonary<br>Bypass: Effect of Different Pharmacological<br>Interventions .....  | 391 |
| W. Riegel, G. Spillner, V. Schlosser, K. Lang,<br>and W. H. Hörl  |     |
| Significant Role of Protease Inhibition by Aprotinin<br>in Myocardial Protection from Prolonged<br>Cardioplegia with Hypothermia .....  | 399 |
| M. Sunamori, R. Innami, H. Fujiwara,<br>M. Yokoyama, A. Suzuki  |     |

|  |     |
|--|-----|
| Fibrinolysis Caused by Cardio-Pulmonary Bypass<br>and Shed Mediastinal Blood Retransfusion -<br>Is it of Clinical Relevance? ..... | 405 |
| W. Dietrich, A. Barankay, P. Wendt,<br>A. Stemberger, G. Blümel, M. Spannagl,<br>M. Jochum, and J. A. Richter                      |     |

## VII PROTEINASES IN CATABOLIC STATES

|   |     |
|---|-----|
| Nutrition and Protease Activity .....   | 411 |
| J. D. Kopple  |     |
| Insulin Degradation after Injury in Man .....   | 421 |
| S. M. Hoare, K. N. Frayn, and R. E. Offord  |     |
| Endotoxin Abolishes the Induction of Alpha-2-Macro-<br>globulin Synthesis in Cultured Human<br>Monocytes Indicating Inhibition of the<br>Terminal Monocyte Maturation into<br>Macrophages ..... | 425 |
| J. Bauer, U. Ganter, and W. Gerok   |     |
| Local and General Defence Mechanisms in Bacterial<br>and Chemical Peritonitis .....   | 433 |
| A. Lassin, M. Delshammer, and K. Ohlsson  |     |
| Deficient Phagocytosis Secondary to Proteolytic<br>Breakdown of Opsonins in Peritonitis Exudate .....   | 441 |
| A. Billing, U. Fröhlich, M. Jochum, and<br>H. Kortmann  |     |
| Proteolysis and Lipid Peroxidation - Two Aspects of<br>Cell Injury in Experimental Hypovolemic-<br>Traumatic Shock .....  | 449 |
| H. Redl, S. Hallström, C. Lieners, W. Fürst,<br>and G. Schlag   |     |
| Plasma Levels of Elastase 1 Protease Inhibitor<br>Complex in the Monitoring of ARDS and Multi-<br>Organ Failure - A Summary of Three Clinical<br>Trials .....                                   | 457 |
| H. Redl, E. Paul, R. J. A. Goris, R. Pacher,<br>W. Woloszczuk, and G. Schlag  |     |
| PMN Elastase and Leukocyte Neutral Proteinase<br>Inhibitor (LNPI) from Granulocytes as<br>Inflammation Markers in Experimental-<br>Septicemia .....   | 465 |
| R. Geiger, S. Sokal, G. Trefz, M. Siebeck,<br>and H. Hoffmann   |     |
| Plasma Derivative Replacement Therapy in<br>Diss.Intravasc.Coag.(DIC) Induced by Septic<br>Disorders with highly Elevated Elastase<br>Alpha-1-AT-Complexes .....                                | 473 |
| R. Egbring, R. Seitz, M. Wolf, K. Havemann,<br>L. Lerch, H. Blanke, and G. Fuchs  |     |



|   |     |
|---|-----|
| Neutrophil Elastase, Thrombin and Plasmin in Septic Shock .....   | 481 |
| R. Seitz, M. Wolf, R. Egbring, and K. Havemann  |     |
| Elastase-Alpha-1-Proteinase Inhibitor: An Early Indicator of Septicemia and Bacterial Meningitis in Childhood .....               | 485 |
| C. P. Speer, M. Rethwilm, and M. Gahr   |     |
| Serum Pancreatic Secretory Trypsin Inhibitor (PSTI) in Seriously Injured and Septic Patients .....                                | 493 |
| H. Tanaka, M. Ogawa, T. Yoshioka, and T. Sugimoto   |     |
| Changes in PMN-Elastase in Blood and in Renal and Plasma Kallikrein-Kinin Systems after Severe Burn Injury .....                  | 499 |
| G. Bönner, W. Niermann, R. Festge, and U. Büchsler  |     |
| Serum Pancreatic Secretory Trypsin Inhibitor (PSTI) in Patients with Inflammatory Diseases .....                                  | 505 |
| M. Ogawa, T. Shibata, T. Niinobu, K. Uda, N. Takata, and T. Mori  |     |
| The Effect of Aprotinin Administration on the Intraoperative Histamine Release and Haemostatic Disorders .....                    | 509 |
| H. Harke and S. Rahman  |     |
| Increased Mortality in Septic Rats after Leupeptin Application .....  | 515 |
| E. Kovats, J. Karner, G. Ollenschläger, J. Karner, A. Simmel, and E. Roth   |     |
| Lysosomal Enzymes and Granulocyte Elastase in Synovial Fluid after Multiple Traumatic Injuries .....                              | 519 |
| M. Hörl and H. P. Bruch   |     |
| A Serine Proteinase Inhibitor in Human Articular Cartilage-Possible Role in the Pathogenesis of Inflammatory Joint Diseases ..... | 523 |
| H. Burkhardt, M. Kasten, and S. Rauls   |     |
| Detection of Granulocyte Elastase Specific IgG Split Products in Rheumatoid Synovial Fluid .....                                  | 531 |
| I. Eckle, G. Kolb, F. Neurath, and K. Havemann  |     |

## VIII PROTEASES AND MALIGNOMA

|   |     |
|---|-----|
| The T Cell Specific Serine Proteinase TSP-1:<br>Biochemical Characterization, Genetic<br>Analysis, and Functional Role .....  | 535 |
| H.-G. Simon, U. Fruth, R. Geiger,<br>M. D. Kramer, and M. M. Simon  |     |
| Pancreatic Secretory Trypsin Inhibitor in<br>Cancer .....   | 547 |
| M. Ogawa, N. Tomita, A. Horii, N. Matsuura,<br>M. Higashiyama, T. Yamamoto, A. Murata,<br>T. Mori, and K. Matsubara   |     |
| Proteases and Antiproteases in Ascites -<br>Differentiation of Malignant and Non-<br>malignant Ascites and Prediction of<br>Coagulopathy in Ascites Retransfusion ..... | 555 |
| J. Schölmerich, E. Köttgen, B. A. Volk, and<br>W. Gerok   |     |
| Alpha-1-Antitrypsin and Alpha-1-Antichymotrypsin<br>Serum Level in Relation to Staging and<br>Postoperative Clinical Course of Human<br>Colorectal Cancer .....         | 561 |
| A. Kuryliszyn-Moskal, K. Bernacka, and<br>S. Sierakowski  |     |
| Inhibition of Proteases During Extracorporeal<br>Extremity Perfusion .....  | 565 |
| H. Walther, H. Müller, and K. R. Aigner   |     |
| INDEX .....   | 569 |

## DEFICIENT PHAGOCYTOSIS SECONDARY TO PROTEOLYTIC BREAKDOWN OF OPSONINS IN PERITONITIS EXUDATE

A. Billing<sup>a</sup>, D. Fröhlich<sup>a</sup>, M. Jochum<sup>b</sup> and  
H. Kortmann<sup>a</sup>

- a) Chirurgische Klinik und Poliklinik der Universität München, Klinikum Großhadern, Marchioninistr. 15, 8000 München 70, Germany
- b) Institut für klinische Chemie und klinische Biochemie der Universität München

### INTRODUCTION

In peritonitis, proper functioning of the intraabdominal local defence system is crucial for a favourable outcome and survival of the patient. Peritonitis exudate is characterized by the presence of a large number of viable bacteria despite a huge population of intact PMN-leukocytes. Although phagocyte function, the main factor of cellular defence, is intact or even stimulated in peritonitis exudate (1,2), there is no adequate explanation of how bacteria can persist in surroundings rich in PMN-leukocytes.

An adequate sufficient intraabdominal host defence results from a balanced cooperation between cellular and fluid phase components. The humoral immune process of recognizing and labelling a microbe as antigenically foreign is described as opsonization. This can proceed via non-specific or specific mechanisms (3). The latter is immunoglobulin G (IgG) dependent. Both pathways result in complement activation which leads to a liberation of opsonins, mediators of inflammation and microbicidal components. The main factors of opsonization are C3-derived complement components and IgG. Physiological C3 activation results in its breakdown into the fragments C3c and C3d. Unspecific proteolytic breakdown of opsonic factors in pleural empyema has also been described (4).

Phagocytosis leads to cell activation and also results in an extracellular release of lysosomal and oxidative granulocyte enzymes (5). Myeloperoxidase is known to impair opsonization (6). Proteolytic and oxidative destruction can destroy biological activity of protein components without altering their antigenicity. Thus, despite immunologically determined high concentrations, there may be a functional deficit in such factors. In parallel to enzyme release, particle attachment leads to a strong activation of oxygen metabolism in phagocytes, resulting in the generation of oxygen-derived free radicals. These micro-

bicidal and cytotoxic substances are known to destroy  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ PI). Using a photometric amplification system, the release of oxygen-derived free radicals can be measured as chemiluminescence (CL) and is assumed to be a quantitative parameter for phagocytic activity. Using a constant number of phagocytes, CL-measurements can be used as a direct parameter for the quality of particle opsonization (7,8).

Little is known about intraabdominal opsonization. We developed a simple CL assay to evaluate opsonic activity (OA) in peritonitis exudates and serum samples of patients with acute and persisting peritonitis. The latter group was treated with Etappenlavage which means planned relaparotomy until clearance of the abdominal cavity. In addition, we investigated opsonin levels as well as released granulocytic proteins.

## MATERIAL AND METHODS

### Patients

50 abdominal exudates and corresponding blood samples were drawn intraoperatively from 27 patients with diffuse purulent peritonitis. Exudates were centrifugated, while blood was processed into serum and EDTA-plasma.

### Chemiluminescence assay for opsonic activity

Zymosan was preopsonized with pooled normal serum, patients' serum or patients' exudate. The final chemiluminescence assay contained 0,05 ml diluted EDTA-blood (1/15) from healthy volunteers, 0,8 ml Veronal buffer and 0,1 ml Luminol solution (9). The reaction was started by adding 0,05 ml of the opsonized zymosan (20 mg/ml). The 30 min. integral of chemiluminescence was calculated. In each assay zymosan opsonized with normal serum, patients' serum and patients' exudate was tested simultaneously. As all other conditions (blood and buffer concentration) were identical, the resulting different chemiluminescence response is due to the quality of opsonization (10). Opsonic activity was expressed as a percentage of the normal serum value.

### Opsonin studies

C3 and IgG levels were measured with a standard radial immunodiffusion assay (Behringwerke Marburg, normal values: IgG 1250 mg/dl and C3 82 mg/dl). C3 splitting was demonstrated by crossed immunoelectrophoresis according to Ganroth (11) employing a C3c antibody (Behringwerke Marburg).

### Tests for PMN-enzymes

Elastase in complex with  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ PI) and myeloperoxidase concentrations were measured by ELISA: plasma reference for complexed elastase: 50-181  $\mu$ g/l, and for myeloperoxidase: 25-47  $\mu$ g/l (12, 13). Free elastase activity was measured with a chromogenic substrate (14) or by adding  $\alpha_1$ PI and then re-assaying elastase- $\alpha_1$ PI complex.

## RESULTS

### Opsonization in serum

In acute peritonitis C3 and IgG serum levels were close to the lower limit of the normal range and were increased in patients with persisting peritonitis (Table 1). Opsonic activity in patients' serum was well correlated with a C3/IgG index which results from addition of C3 and IgG concentrations. Computerized correlation analysis resulted in a S-shaped curve, very similar to a dilution curve of normal serum (Fig. 1). In Fig. 1 serum samples of patients (n=23) with acute and persisting peritonitis at the time of sample collection were included.

Table 1. Opsonin levels (IgG, C3) and opsonic activity (OA) in patient serum (% of normal  $\pm$  standard deviation)

|     | acute peritonitis<br>(n=13) | persisting peritonitis<br>(n=14) |
|-----|-----------------------------|----------------------------------|
| IgG | 62.8 $\pm$ 29.3             | 109.1 $\pm$ 30.8                 |
| C3  | 65.3 $\pm$ 23.1             | 83.0 $\pm$ 21.6                  |
| OA  | 85.8 $\pm$ 33.5             | 115.4 $\pm$ 20.8                 |

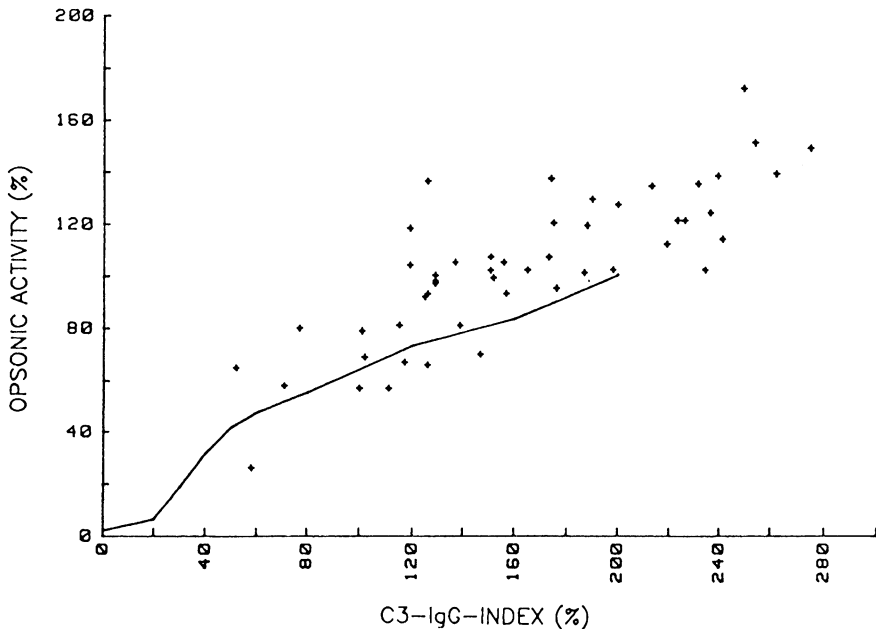


Fig. 1. Correlation of opsonic activity and opsonin concentration in patient serum. The C3-IgG-index results from addition of serum concentrations of both parameters. The curve demonstrates the correlation from serial dilutions of normal serum (1:2 to 1:10).

### Opsonization in exudate

In peritonitis exudates, mean protein content was 66 % of

serum levels in acute peritonitis and 62 % of serum levels in Etappenlavage. The electrophoretic protein distribution pattern was similar to serum, indicating peritoneal permeability even for large molecules. Opsonin concentrations are listed in Table 2. According to the serum correlation of opsonin concentration and function, these opsonin levels should result in an opsonic activity of 58 % of normal in acute peritonitis and 56 % of normal in Etappenlavage. The experimental determination of opsonic function, however, showed a much lower activity (8.4 % and 4.6 % of normal, respectively) indicating a pronounced deficit in particle opsonization in peritonitis exudates.

Table 2. Opsonin levels (IgG, C3) and opsonic activity (OA) in peritonitis exudates (% of normal serum value  $\pm$  standard deviation).  $OA_{exp}$  is the expected OA according to the correlation between IgG/C3 and OA in serum,  $OA_{real}$  is the actual OA in exudate.

|             | acute peritonitis<br>(n=13) | Etappenlavage<br>(n=14) |
|-------------|-----------------------------|-------------------------|
| IgG         | 43.9 $\pm$ 21.3             | 52.9 $\pm$ 18.1         |
| C3          | 35.8 $\pm$ 27.8             | 25.5 $\pm$ 6.8          |
| $OA_{exp}$  | 58                          | 56                      |
| $OA_{real}$ | 8.4                         | 4.6                     |

To evaluate opsonin breakdown, crossed immunoelectrophoresis was carried out in 6 patients' serum and exudate samples employing a C3c antibody. In peritonitis serum only a small amount of C3 was fragmented (Fig. 2b). In exudate, however, depending on the leukocyte concentration, a great part (Fig. 3c) or almost all (Fig. 3d) C3 was split into fragments of lower molecular weight. Thus, the opsonic deficit in purulent exudates was accompanied by an extensive breakdown of the complement factor C3.

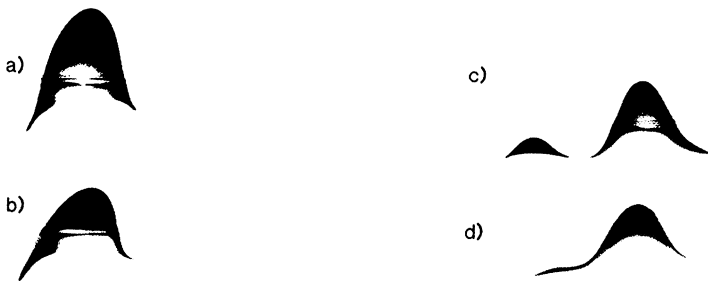


Fig. 2. Crossed immunoelectrophoresis for C3 in serum and exudate.

- a) Normal serum, no C3 splitting
- b) Patient serum, only trace amounts of C3 breakdown products
- c) Exudate (22,000 leukocytes/mm<sup>3</sup>), pronounced C3 splitting into smaller components (right peak)
- d) Purulent peritonitis exudate (110,000) leukocytes/mm<sup>3</sup>), almost complete breakdown of C3

Unspecific proteolytic and oxidative activity in peritonitis exudate

In 27 exudates, we quantified complexed PMN-elastase and myeloperoxidase levels. Elastase concentrations were elevated up to 250 mg/l, which is 2000 times higher than the normal plasma range. We also found extremely high concentrations for myeloperoxidase, reaching up to 160 mg/l (Table 3).

Table 3. Complexed PMN-elastase and myeloperoxidase levels in peritonitis exudates (mean  $\pm$  standard deviation,  $\mu$ g/l).

|                            | acute peritonitis<br>(n=13) | Etappenlavage<br>(n=14) |
|----------------------------|-----------------------------|-------------------------|
| elastase ( $-\alpha_1$ PI) | 75,972 $\pm$ 52,366         | 89,853 $\pm$ 67,570     |
| myeloperoxidase            | 34,458 $\pm$ 42,661         | 55,402 $\pm$ 39,005     |

In several exudates we could demonstrate free elastase activity, both with a specific chromogenic substrate and an  $\alpha_1$ PI-binding assay. In some exudates up to 70 % of the total elastase content was found to be uninhibited free elastase. The concentration for  $\alpha_1$ -proteinase inhibitor in these exudates ranged from 99 to 341 mg/dl, which, calculated on the basis of the molar ratio of inhibitor concentration versus proteinase, should be sufficient for complete elastase inhibition. In patients with gastrointestinal perforation, intraabdominal elastase levels varied within a wide range (Table 4).

Table 4. Elastase (in complex or active) and  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ PI) in peritonitis exudates.

|        | $\alpha_1$ PI-elastase<br>complex(mg/l) | free elastase<br>(mg/l) | total elastase<br>(mg/l) | total $\alpha_1$ PI<br>(mg/l) |
|--------|---|-------------------------|--------------------------|-------------------------------|
| Pat. 1 | 120.6                                   | 272.3                   | 392.9                    | 2250                          |
| Pat. 2 | 45.7                                    | 0.5                     | 46.2                     | 1610                          |
| Pat. 3 | 111.1                                   | 1.6                     | 112.7                    | 1060                          |
| Pat. 4 | 54.6                                    | 73.1                    | 127.7                    | 990                           |

To investigate the possible influence of proteolytic lysosomal enzymes on opsonic activity we compared both parameters. Correlation of the opsonic deficit with elastase levels in exudates revealed that only exudates with a low concentration of complexed elastase ( $<10$  mg//l) reached an almost normal opsonic activity, whereas exudates with high elastase concentrations were deficient in opsonic function (Fig. 3).

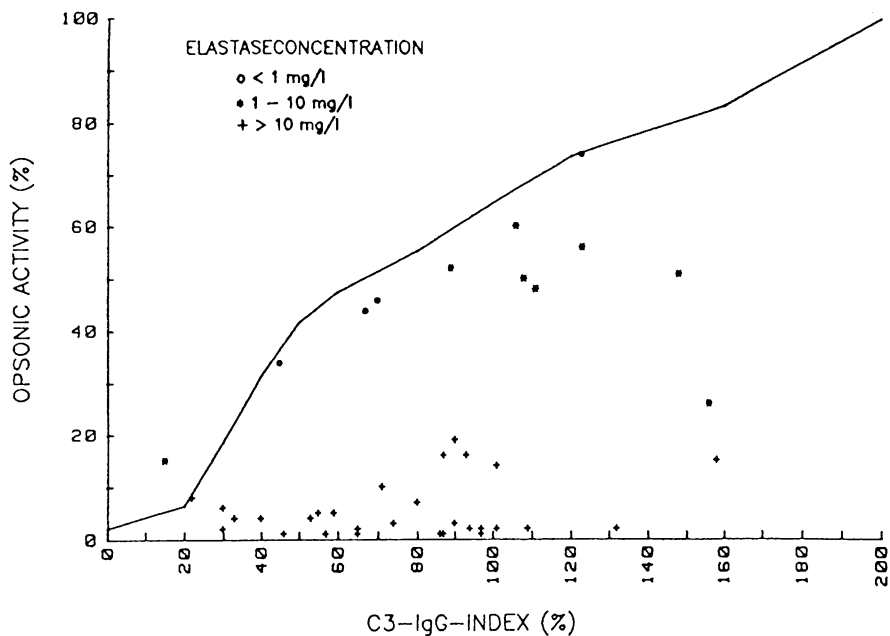


Fig. 3. Correlation of opsonic activity and elastase concentration in peritonitis exudates. The curve demonstrates the relation between opsonin concentration and opsonic activity. Only exudates with elastase concentrations  $\leq 10$  mg/l reveal adequate opsonic activity.

#### DISCUSSION

The CL-approach described here, provides a rapid, reliable non-destructive method for quantitative analysis of opsonic capacity in serum and exudate. Thereby, in normal as well as in patient serum the key role of IgG and C3 for opsonization could be confirmed.

Little information is available about intraperitoneal fluid-phase defence activity. In pleural empyema, deficient phagocytosis due to breakdown of opsonins has been described (4). We could demonstrate a high peritoneal permeability in peritonitis giving way even for large proteins. Despite sufficient immunologically measurable opsonin levels our results revealed an extended dysfunction of particle opsonization in human peritonitis exudates. Most of the immunologically found opsonins were functionally destroyed. The crossed immunoelectrophoresis gave evidence that purulent peritonitis exudates contained hardly any intact physiologically active C3. The identified C3 fragments seem to be degraded products without opsonic function.

In exudates we found extremely high levels of PMN-elastase (most of it in complex with  $\alpha_1$ PI) and myeloperoxidase, indicating the release of a major part of the total phagocytic enzyme content. Due to a slow peritoneal clearance of elastase- $\alpha_1$ PI complexes, enzyme concentrations are further increased. Despite an immunologically sufficient concentration of  $\alpha_1$ PI we could demonstrate free elastase activity in some exudate samples. Oxidative impairment of  $\alpha_1$ PI has been described (15)



and may be due to the release of myeloperoxidase and highly reactive oxygen products during phagocytosis.

For the first time these data reveal clearly that the dysfunction of the intraabdominal defence system in acute peritonitis results from impaired opsonic capacity. One major underlying pathomechanism may be oxidative inactivation of the  $\alpha_1$ -proteinase inhibitor thus allowing unspecific proteolytic opsonin degradation by free lysosomal enzymes. For further improvement in therapy the effect of local proteinase-inhibitor application has to be considered.

### Acknowledgment

We thank Dipl.-Ing. B. Schmidt from the nephrology research lab (Med. Klinik I der Universität München) for the performance of the crossed immunoelectrophoresis.

### REFERENCES

- 1) J. Freischlag, B. Backstrom, D. Kelly, G. Keehn, B. a. R. Busuttill  
Comparison of blood and peritoneal neutrophil activity in rabbits with and without peritonitis  
J. of Surg. Res.; 40: 145-151, 1986
- 2) A. Billing, H. Kortmann  
Nachweis zellulärer und humoraler Abwehrdefekte bei der eitrigen Peritonitis mit einem modifizierten Chemilumineszenzverfahren  
Acta chir. Austriacae 3: 340-341, 1986
- 3) H. Hahn  
Mechanismen der körpereigenen Infektabwehr  
FAC, Band 3-2, 139-150, 1984
- 4) F. A. Waldvogel, P. Vaudaux, P. D. Lew, A. Zwahlen, S. Suter, U. Nydegger  
Deficient phagocytosis secondary to breakdown of opsonic factors in infected exudates  
Adv. Exp. Med. and Biol. 141: 603-610, 1984
- 5) K. Ohlsson, I. Olsson  
The extracellular release of granulocyte collagenase and elastase during phagocytosis and inflammatory processes  
Scand. J. Haematol. 19: 145-152, 1977
- 6) B. I. Coble, C. Dahlgren, J. Hed, O. Stendhal  
Myeloperoxidase reduces the opsonizing activity of immunoglobulin G and complement component C3b  
Biochem. et Biophys. Acta 802: 501-505, 1987
- 7) P. Bellavite, P. Dri, V. Della Bianca, M. C. Serra  
The measurement of superoxide anion production by immunoglobulin G and complement component C3b  
Europ. J. Clin. Invest. 13: 363-368, 1983
- 8) R. C. Allen, M. Lieberman  
Kinetic analysis of microbe opsonification based on stimulated polymorphnuclear leukocyte oxygenation activity  
Inf. and Imm. 45: 475-482, 1984

- 9) D. Inthorn, Th. Szczeponik, B. Mühlbayer, M. Jochum, H. Redl  
 Studies of granulocyte function (chemiluminescence response) in postoperative infection. In: Schlag, G., Redl, H. eds.; First vienna shock forum, Part B.  
Progr.in Clin.and Biol.Res. 263B: 51-58, 1987
- 10) R.C. Allen, M.M. Liebermann  
 Kinetic analysis of microbe opsonification based on stimulated polymorphonuclear leukocyte oxygenation activity  
Inf. and Imm. 45: 475-482, 1984
- 11) P.O. Ganroth  
 Crossed immunoelectrophoresis  
Scand.J.Clin.Lab.Invest. 29: 39-41, 1972
- 12) S. Neumann, G. Gunzer, N. Henrich, H. Lang  
 PMN-elastase assay: enzyme immunoassay for human polymorphonuclear elastase complexed with  $\alpha_1$ -proteinase inhibitor  
J.Clin.Chem.Clin.Biochem. 22: 693-697, 1984
- 13) S. Neumann, G. Gunzer, H. Lang, M. Jochum, H. Fritz  
 Quantitation of myeloperoxidase from human granulocytes as an inflammation marker by enzyme-linked immunosorbent assay  
Fresenius Z.Anal Chem. 324: 365, 1986
- 14) M. Jochum, A. Bittner  
 Inter- $\alpha$ -trypsin inhibitor of human serum: an inhibitor of polymorphonuclear granulocyte elastase  
Hoppe Seyler's Z.Physiol.Chem. 364: 1709-1715, 1983
- 15) N.R. Matheson, P.S. Wong, J. Travis  
 Enzymatic inactivation of human  $\alpha_1$ PI by neutrophil myeloperoxidase  
Biochem.Biophys.Res.Comm. 88: 402, 1979



## INDEX

- Acid phosphatase, 412  
Acid protease, 239  
Acute phase reactants, 171  
Acute renal failure, 309, 323,  
339, 345, 361  
Acute uremia, 331  
Acylation, 161  
Adrenalectomy, 257  
Adult respiratory distress  
syndrome, 17, 149  
Affinity chromatography, 36  
AIDS, 3  
Alanine aminopeptidase, 351  
Alkaline protease, 239, 326,  
334  
Alpha<sub>1</sub>-  
antichymotrypsin, 97, 171,  
561  
antitrypsin, 145, 473, 496,  
505, 561  
inhibitor, 191  
macroglobulin, 183  
protease inhibitor, 346,  
434, 555  
proteinase, 405  
proteinase inhibitor, 42,  
97, 107, 120, 123,  
171, 385, 393  
Alpha<sub>2</sub>-  
antiplasmin, 127, 434, 473,  
515, 555  
macroglobulin, 127, 145,  
171, 183, 191, 199,  
346, 425, 434, 496,  
500, 555  
Alpha-D-galactosidase, 46  
Alpha-D-glucosidase, 46  
Alpha-macroglobulins, 183  
Alveolar-arterial oxygen  
tension difference, 151  
Aminopeptidase A, 278  
Aminopeptidase M, 279  
Angiotensin converting enzyme,  
13, 145  
Angiotensinase A, 280  
Angiotensinogen, 7  
Antiplasmin, 405  
Antithrombin III, 127, 555  
Aprotinin, 399, 509, 542, 566  
Arachidonic acid metabolites,  
226  
Arginylation, 159  
Articular cartilage, 523  
Arylsulfatase, 412, 520  
Ascites, 555  
Aspartic proteinase, 1  
Astrocytes culture, 200  
Atrial natriuretic factor, 16

Autophagy, 166  
 Azocasein, 141  
  
 Beta-galactosidase, 412  
 Beta-glucuronidase, 399, 412, 520  
 Beta-1-anticollagenase, 42  
 Beta-D-glucuronidase, 46  
 Bioincompatibility, 365  
 Bowman-birk-inhibitor, 110  
 Bradykinin, 15  
 Bronchial proteinase inhibitor, 115  
 Bronchoalveolar lavage, 137, 351  
 Burn injury, 499  
  
 Calpain, 175  
 Cancer, 8, 547  
 Captopril, 13  
 Carboxypeptidase, 160  
 Cardioplegia, 399  
 Cardiopulmonary bypass, 391, 405  
 Cartilage inhibitor, 536  
 Casein, 6  
 Catabolic factors, 315  
 Cathepsin  
   A, 160  
   B, 243, 245, 285, 306, 413  
   D, 3, 412, 520  
   E, 3  
   G, 23, 98, 123, 474, 525  
   L, 289, 306  
 Chemiluminescence, 57, 378, 442  
 Chronic renal failure, 345, 361  
 Chymase, 133  
 Chymosin, 3  
  
 Chymotrypsin, 75  
   A, 97  
   -like proteases, 97  
 Chymotrypsin inhibitor, 134  
 C<sub>1</sub>-inhibitor, 434  
 Coagulopathy, 557  
 Collagen, 33  
 Collagenase, 33  
 Colon, 103  
 Colorectal cancer, 561  
 Complement, 365  
   components, 226  
 Cuprophane, 373, 377  
 Cyclosporin A, 293  
 Cysteine proteinases, 161, 175, 215  
 Cytochrome C, 378  
 Cytokines, 177  
 Cytolysis, 539  
 Cytolytic activity, 539  
 Cytolytic T lymphocyte line, 535  
  
 Dermis, 89  
 Digestion products, 289  
 Dipeptidyl peptidase, 351  
   IV, 207  
 Dipeptidylaminopeptidase, 160  
   IV, 278  
 Domain, 175  
 Duodenum, 103  
  
 Ectoexopectidase, 45  
 Effective respiratory compliance, 151  
 Eglin C, 331  
 Elastase, 23, 42, 57, 149, 346, 378, 385, 392, 445, 457, 465, 474, 481, 485, 519, 531

Elastinolysis, 65  
 Electron micrographs, 300  
 Emphysema, 107  
 Enalapril, 13  
 Endoaminopeptidase, 160  
 Endocytic vacuoles, 289, 305  
 Endopeptidase, 13, 160, 161, 351  
 Endotoxin, 425, 474  
 Enkephalinase, 13  
 Epidermis, 89  
 Exogen allergic alveolitis, 137  
 Exoglycosidases, 45  
 Exopeptidase, 160  
 Extracorporeal extremity perfusion, 565  
  
 Factor H, 373  
 Fibrinolysis, 405  
 Fibrin(ogen) degradation products, 405  
 Fibronectin, 347, 555  
  
 Gamma-glutamyl transpeptidase, 47, 276, 351  
 Gas exchange, 150  
 Gastric mucosa, 102  
 Gastricsin, 1  
 Gelatinase, 33  
 Gelfiltration, 36  
 Gentamicin, 305  
 Glomerular disease, 267  
 Glomerular proteases, 275  
 Glomerulonephritis, 280  
 Glucocorticoids, 45, 257  
 Glutamyl aminopeptidase, 351  
 Granulocytes, 465  
  
 H-D-Pro-Phe-Arg-chloromethyl ketone, 540  
  
 Hemodialysis, 245, 377  
 Hepatocytes, 159, 176, 186, 191  
 Hepatocyte-stimulating factor, 191  
 Hepatome, 207  
 Heterolysosomes, 284, 305  
 High molecular mass proteinases, 216  
 Histamine, 133, 509  
 Histochemical toxicology, 45,  
 Histochemistry, 351  
 Human proteinase inhibitor, 123  
 Human secretory leukocyte protease inhibitor, 123  
 Hydrophobicity, 159  
 Hydroxyproline, 145  
 Hypothermia, 399  
  
 Idiopathic pulmonary fibrosis, 137  
 IgG split products, 531  
 Immunohistochemical staining, 211  
 Immunolocalization, 210  
 Immunosuppression, 385  
 Inflammation, 465  
 Inflammatory diseases, 505  
 Inflammatory joint diseases, 523  
 Injury severity score, 422  
 Insulin, 421  
     receptor, 239  
     resistance, 315  
 Interferon  $\beta$ , 191  
 Interleukin-1, 191, 225  
 Interstitial lung disease, 137  
 Interstitial lung fibrosis, 145

Inter-alpha-trypsin-inhibitor, 75, 89  
 Kallikrein, 172, 453, 499  
 Kidney, 275, 295  
     sections, 277  
     transplantation, 309  
 Kininase, 13  
 Kininogens, 175, 361, 501  
 Kupffer cells, 193  
 Kwashiorkor, 413  
  
 Laminin, 268  
 Leukocyte elastase, 107, 115, 128, 435  
 Leupeptin, 324, 515  
 Long chain fatty acids, 216  
 Lung elastin, 107, 115  
 Lysosomal  
     enzymes, 519  
     hydrolases, 236  
     proteases, 283, 305  
 Lysosomes, 160, 284, 305  
 Lysozyme, 226  
  
 Macrophages, 193, 425  
 Magnesium, 299  
 Malignant tumor, 551  
 Malnutrition, 411  
 Marasmus, 413  
 Mast cells, 133  
 Melanoma, 566  
 Meningitis, 485  
 Meprin, 293  
 Metabolic acidosis, 318  
 Metalloendopeptidase, 13  
 Metalloproteinase, 33, 293  
 Methylcasein, 217  
 Monoclonal antibodies, 207  
 Monocytes, 193, 425  
  
 Monocyte-derived factor, 191  
 Monokines, 226  
 Mononuclear phagocytes, 225  
 Multicatalytic proteinase, 216  
 Muscle protein turnover, 225  
 Muscle proteolysis, 318  
 Myofibrillar proteinase, 263  
  
 N-acetylglucosaminidase, 402, 413, 450  
 N-end rule, 161  
 Neopterin, 461  
 Nephrotoxic nephritis, 267  
 Neurotensin, 15  
 Neutral proteases, 226  
 Neutrophil elastase, 27, 28, 65, 75, 123  
 Nitrogen balance, 323  
 Non-lysosomal proteases, 235  
 N<sup>t</sup>-methyl-histidine, 262, 328, 336  
 Nutrition, 411  
  
 Oleic acid, 219  
 Oleoyl-coenzyme A, 220  
 Opsonins, 441  
 Ouchterlony double diffusion assay, 185  
 Overnutrition, 415  
 Oxygen radical, 474  
 Oxytocin, 15  
  
 Pancreatic elastase, 27  
 Pancreatic elastase 2, 97  
 Pancreatic secretory trypsin inhibitor, 101, 493, 505, 547  
 Pancreatitis, 245  
 Papain, 161, 175

Parathyroid hormone, 257  
 Parathyroidectomy, 257  
 Penicillinopepsin, 5  
 Pepsinogen, 1  
 Pepsins, 1  
 Peritonitis, 433, 441  
 Peroxidation, 449  
 Phosphatases, 45  
 Phosphatidyl-choline, 220  
 Phosphatidyl-D, L-glycerol, 220  
 Phosphatidyl-inositol, 113  
 Phospholipase C, 212  
 Phosphoramidon, 13  
 Physical exercise, 57  
 Plasmin, 172, 473, 481  
 Plasminogen, 347, 405, 512, 555  
 PMN-Elastase, 499, 525  
 Pneumonia, 19  
 Polyguanylic acid, 65  
 Polynucleotids, 65  
 Polyribosylribitol  
   phosphatase, 73  
 Prekallikrein, 347, 500  
 Preproelastase, 27  
 Prostaglandin E2, 225  
 Protease, 238  
 Protein degradation, 317  
 Protein split products, 339  
 Proteinuria, 283, 267  
 Proximal tubule, 283  
 Proximal tubule cells, 300  
 Pulmonary vascular  
   resistance, 151  
 Rat  
   brain, 199  
   liver, 199, 207  
 Reactive oxygen, 226  
 Renin, 3, 361  
 Respiratory tract, 123  
 Rheumatoid  
   arthritis, 107  
   joint destruction, 526  
   synovial fluid, 531  
 RNA homopolymers, 71  
 RNase, 520  
 Salicylic acid, 45  
 Sarcoidosis, 137, 145  
 Seminal plasma inhibitor, 116  
 Sepsis, 309, 339, 494, 517  
 Septicemia, 485  
 Septic shock, 481  
 Sequence homology, 27  
 Serine  
   endopeptidase, 536  
   proteinase, 535  
   proteinase inhibitor, 523  
 Serpins, 174  
 Shock, 449, 481  
 Skeletal muscle, 215, 243  
 Skin, 89  
 Starvation, 411  
 Stearoyl-coenzyme A, 220  
 Stearoyl-L-carnitine, 220  
 Substance P, 15  
 Synovial fluid, 519  
 Thiorphan, 13  
 Thrombin, 473, 481  
 T lymphocytes, 539  
 Trauma, 309, 339  
 Traumatic injuries, 519  
 Trichloroacetic acid, 339  
 Trypsin, 75, 525  
 Trypsin inhibitor, 134



Tryptase, 133  
Tubular proteases, 275  
Tubule lumen, 289, 305  
Tumor necrosis factor, 191  
Turnover, 160  
Type IV collagen, 268  
Tyrosine, 240  
  
Ubiquitination, 161  
Ulcers, 8  
  
Urea-N appearance, 259  
Uremia, 315  
Uremic  
    catabolism, 323  
    patients, 246  
    rats, 257  
Urinary proteinase activity,  
    309  
Urinary proteinases, 267