

European Journal of Clinical Investigation

The Journal of The European Society for Clinical Investigation

Editors-in-Chief A. M. McGregor, J. Moxham, A. L. W. F. Eddleston

Volume 24, 1994

4 Med. 67 93

*24
1994*

1-500

Published by Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, UK.

© 1994 Blackwell Scientific Publications Ltd. Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Blackwell Scientific Publications Ltd for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of \$10.50 per copy is paid directly to CCC, 222 Rosewood Drive, Suite 910, Danvers, MA 01923, USA. This consent does not extend to other kinds of copying, such as copying for general distribution for advertising or promotional purposes, for creating new collective works or for resale. Special requests should be addressed to the Editor 0014-2972/94 \$10.50.

The use of registered names, trade marks, etc., in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulation and therefore free for general use.

Published by Blackwell Scientific Publications Ltd
Osney Mead, Oxford, OX2 0EL, UK.

25 John Street, London, WC1N 2BL, UK.

23 Ainslie Place, Edinburgh, EH3 6AJ, UK.

238 Main Street, Cambridge, MA 02142, USA.

54 University Street, Carlton, Victoria 3053, Australia.

Arnette SA, 1 rue de Lille, 75006, France.

Blackwell Wissenschaft Verlag GmbH, Kurfürstendamm 57, D-10707, Germany.

Blackwell MZV, Feldgasse, A-1238 Wien, Austria.



ISSN 0014-2972

Printed in Great Britain at The Alden Press Ltd, Oxford.

Contents of Volume 24

Number 1, January 1994

Review: The fragile X syndrome: implications of molecular genetics for the clinical syndrome
F. Rousseau

1

Review: Viruses and auto-immune hepatitis
B. Rehermann, K. Michitaka, M. Durazzo, K Mergener, P. Velev and M. P. Manns

11

Commentary: Lithotripsy of gallbladder stones

T. Sauerbruch and M. Neubrand

20

Brittleness of gallstones to lithotripsy: effect of physicochemical and ultrastructural characteristics

G. Choudhuri, D. K. Agarwal, R. V. Phadke, V. Ramesh, W. Hauser, J. Kumar, T. S. Negi and A. Kulshreshiha

22

Local serum application: restoration of sufficient host defense in human peritonitis

A. G. Billing, D. Fröhlich, G. Konecny, F. W. Schildberg, W. Machleidt, H. Fritz and M. Jochum

28

Identification of defective binding of low density lipoprotein by the U937 proliferation assay in German patients with familial defective apolipoprotein B-100

C. K. Schewe, H. Schuster, S. Hailer, G. Wolfram, C. Keller and N. Zöllner

36

Proteolytic inactivation of alpha-1-antitrypsin by human neutrophils: involvement of multiple and interlinked cell responses to phagocytosable targets

L. Ottonello, P. Dapino, M. Scirocco, F. Dallegri and C. Sacchetti

42

Circulating somatostatin-28 is not a physiologic regulator of gastric acid production in man

P. Hildebrand, J. W. Ensink, J. Buettiker, J. Drewe, B. Burckhardt, K. Gyr and C. Beglinger

50

Plasma and urinary leukotrienes in sickle cell disease: possible role in the inflammatory process

B. O. Ibe, J. Kurantsin-Mills, J. Usha Raj and L. S. Lessin

57

Human leucocyte-endothelial interactions in peripheral arterial occlusive disease

G. Ciuffetti, R. Lombardini, R. Paltriccia, L. Santambrogio and E. Mannarino

65

Platelet glycoprotein IIb-IIIa and size are increased in acute myocardial infarction

H. Giles, R. E. A. Smith and J. F. Martin

69

Rapid Communication: Serum levels of interleukin-6 and tumour-necrosis-factor-alpha are not correlated to disease activity in patients with rheumatoid arthritis after treatment with low-dose methotrexate

T. C. Wascher, J. Hermann, R. Brezinschek, H. P. Brezinschek, M. Wilders-Truschnig, F. Rainer and G. J. Krejs

73

Rapid Communication: A nitric oxide donor improves uterine artery diastolic blood flow in normal early pregnancy and in women at high risk of pre-eclampsia

B. Ramsay, A. de Belder, S. Campbell, S. Moncada and J. F. Martin

76

Instructions to authors

Number 2, February 1994

Review: Neurobiological aspects of the chronic fatigue syndrome

J. Bearn and S. Wessely

79

Review: Somatostatin and the immune and haematopoietic system; a review

P. M. van Hagen, E. P. Krenning, D. J. Krenning, D. J. Kwekkeboom, J. C. Reubi, P. J. Anker-Lugtenburg, B. Löwenberg and S. W. J. Lamberts

91

Commentary: Role and evaluation of megakaryocytes and platelets in cardiovascular disease. A meeting perspective

C. W. Jackson

100

Decreased clearance of uraemic and mildly carbamylated low-density lipoprotein

S. Hörkkö, K. Huttunen, K. Kervinen and Y. Antero Kesäniemi

105

The diagnosis of pulmonary tuberculosis by gas chromatographic detection of tuberculostearic acid using flame ionization detectors

A. Herz, M. Leichsenring, M. Felten, O. J. Oosthuizen, E. Mayatapek, W. Haas and H. J. Bremer

114

T-cell receptor gene rearrangements in lymphoid and non-lymphoid leukaemias

S. Casares, J. M. Rodriguez, A. Martin and A. Parrado

119

Plasma fibrinogen in relation to serum insulin, smoking habits and adipose tissue fatty acids in healthy men <i>M. Cigolini, G. Targher, G. De Sandre, M. Muggeo and J. C. Seidell</i>	126	Acute dexfenfluramine administration normalizes glucose tolerance in rats with insulin-deficient diabetes <i>R. Arora, S. Dryden, P. E. McKibbin and G. Williams</i>	182
Alpha-subunit and human chorionic gonadotropin- β immunoreactivity in patients with malignant endocrine gastroenteropancreatic tumours <i>M. Grossmann, M. E. Trautmann, S. Poertl, R. Hoermann, P. Berger, R. Arnold and K. Mann</i>	131	Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism <i>R. P. F. Dullaart, W. J. Sluiter, L. D. Dikkeschei, K. Hoogenberg and A. Van Tol</i>	188
Regulation of VLDL secretion in primary culture of rat hepatocytes: involvement in cAMP and cAMP-dependent protein kinases <i>Ó. G. Björnsson, J. D. Sparks, C. E. Sparks and G. F. Gibbons</i>	137	Altered body composition and fuel metabolism in stable kidney transplant patients on immunosuppressive monotherapy with cyclosporine A <i>R. L. Mathieu, J. P. Casez, Ph. Jaeger, A. Montandon, E. Peheim and F. F. Horber</i>	195
Number 3, March 1994		The association between rifamycin-SV (R-SV) related hyperbilirubinaemia and antipyrine clearance as a new test of liver function in cirrhosis <i>M. Persico, M. Romano, N. Villano, F. Montella and S. Gentile</i>	201
Zinc and magnesium in liver cirrhosis <i>E. Rocchi, P. Borella, A. Borghi, F. Paolillo, M. Pradelli, F. Farina and G. Casalgrandi</i>	149	Increase in plasma 3,4-dihydroxyphenylalanine (DOPA) appearance rate after inhibition of DOPA decarboxylase in humans <i>E. Eldrup, M. Lund Hetland and N. Juel Christensen</i>	205
Auditory brain-stem responses (ABRs) in sleep respiratory disorders <i>J. Paquereau, J. C. Meurice, J. P. Neau, P. Ingrand and F. Patte</i>	156	Sex- and age-dependency of IgG auto-antibodies against IL-1 α in healthy humans <i>M. B. Hansen, M. Svenson, K. Abell, K. Varming, H. P. Nielsen, A. Bertelsen and K. Bendtzen</i>	212
Studies on the effects of growth hormone administration <i>in vivo</i> on the rates of glucose transport and utilization in rat skeletal muscle <i>G. Dimitriadis, M. Parry-Billings, B. Leighton, T. Piva, D. Dunger, P. Calder, J. Bond and E. Newsholme</i>	161	Number 4, April 1994	
Na ⁺ /H ⁺ antiporter activity in peripheral blood lymphocytes of obese and type 2 diabetic patients is increased only in the presence of arterial hypertension <i>D. Ghigo, P. Alessio, S. Burzacca, C. Costamagna, G. Anfossi, F. Cavalot, A. Bosia and M. Trovati</i>	166	Review: Clinical complementology: recent progress and future trends <i>B. P. Morgan</i>	219
Enhanced cellular metabolism of very low density lipoprotein by simvastatin. A novel mechanism of action of HMG-CoA reductase inhibitors <i>E. Sehayek, E. Butbul, R. Avner, H. Levkovitz and S. Eisenberg</i>	173	Ascorbic acid reduces the endotoxin-induced lung injury in awake sheep <i>A. Dwenger, H. C. Pape, C. Bantel, G. Schweitzer, K. Krumm, M. Grotz, B. Lueken, M. Funck and G. Regel</i>	229
The relationship of serum total sialic acid with serum acute phase proteins and lipoprotein (a) in patients with severe hypertriglyceridaemia <i>M. Crook, S. Haq, M. Haq and P. Tutt</i>	179	Dietary supplementation with very long-chain n-3 fatty acids in man decreases expression of the interleukin-2 receptor (CD25) on mitogen-stimulated lymphocytes from patients with inflammatory skin diseases <i>E. Søyland, T. Lea, B. Sandstad and A. Devon</i>	236

Large platelets continue to circulate in an activated state after myocardial infarction <i>H. P. Schultheiß, D. Tschöepe, J. Esser, B. Schwippert, P. Rosen, H. K. Nieuwenhuis, C. Schmidt-Soltau and B. Strauer</i>	243	Inhibitory effect of vitamins C and E on the oxygen free radical production in human polymorphonuclear leucocytes <i>K. Herbaczyńska-Cedro, M. Wartanowicz, B. Panczenko-Kresowska, K. Cedro, B. Kłosiewicz-Wąsek and W. Wąsek</i>	316
Systemic lupus erythematosus is associated with increased auto-antibody titers against calreticulin and Grp94, but calreticulin is not the Ro/SS-A antigen <i>J. Boehm, T. Orth, P. Van Nguyen and H.-D. Söling</i>	248	Effects of systemic complement activation on renal circulation of rats <i>K. Schlottmann, E. Gulbins, E. W. Rauterberg and M. Steinhausen</i>	320
Peripheral, rather than hepatic, insulin resistance and atherogenic lipoprotein phenotype predict cardiovascular complications in NIDDM <i>R. Nosadini, E. Manzato, A. Solini, P. Fioretto, E. Brocco, S. Zambon, A. Morocutti, M. Sambataro, M. Velussi, M. R. Cipollina and G. Crepaldi</i>	258	Smoking attenuates the vasoconstrictor response to noradrenaline in type I diabetic patients and normal subjects: possible relevance to diabetic nephropathy <i>C. W. Bodmer, D. T. Valentine, E. A. Masson, M. W. Savage, D. Lake and G. Williams</i>	331
Variable patterns of atrial natriuretic peptide secretion in man <i>A. M. Nugent, G. N. Onuoha, D. J. McEaney, I. C. Steele, S. J. Hunter, K. Prasanna, N. P. S. Campbell, C. Shaw, K. D. Buchanan and D. P. Nicholls</i>	267	Effects of insulin-like growth factor-I (IGF-I), insulin and combined IGF-I-insulin infusions on protein metabolism in dogs <i>A. M. Umpleby, F. Shojaee-Moradie, M. J. Thomason, J. M. Kelly, A. Skottner, P. H. Sonksen and R. H. Jones</i>	337
Phosphotyrosine protein profiles in monocytes after insulin and IGF-I stimulation <i>G. Zoppini, P. Galante, M. Zardini and M. Muggeo</i>	275	Circulating human megakaryocytes in cardiac diseases <i>E. C. M. van Pampus, P. C. Huijgens, A. Zevenbergen, F. W. A. Verheugt and M. M. A. C. Langenhuijsen</i>	345
Strain gauge plethysmography and Doppler ultrasound in the measurement of limb blood flow <i>L. C. M. Pallarés, C. R. Deane, S. V. Baudouin and T. W. Evans</i>	279	Effect of endogenous organic hyperinsulinaemia on blood pressure and serum triglycerides <i>R. Vettor, P. Mazzonetto, C. Macor, C. Scandellari and G. Federspil</i>	350
Association between biosynthesis of nitric oxide and changes in immunological and vascular parameters in patients treated with interleukin-2 <i>D. Miles, L. Thomsen, F. Balkwill, P. Thavasu and S. Moncada</i>	287	Enhancement of cyclosporin A induced hepato- and nephrotoxicity by glutathione depletion <i>G. Inselmann, H. U. Lawerenz, U. Nellessen and H. T. Heidemann</i>	355
Letter to the Editor	291	Lysine-binding species of lipoprotein(a) in coronary artery disease <i>I. Karmansky, H. Shnaider, A. Palant and N. Gruener</i>	360
Number 5, May 1994		Number 6, June 1994	
Review: Neuropeptide Y and energy balance: one way ahead for the treatment of obesity? <i>S. Dryden, H. Frankish, Qiong Wang and G. Williams</i>	293	Commentary: Cholecystokinin as a physiological regulator of gastric acid secretion in man <i>A. H. Gibbons and J. Calam</i>	367
HLA-DPB1 polymorphisms on the MHC-extended haplotypes of families of patients with Graves' disease: two distinct HLA-DR17 haplotypes <i>S. Ratanachaiyavong and A. M. McGregor</i>	309	Cholecystokinin is a physiological regulator of gastric acid secretion in man <i>B. Burckhardt, F. Delco, J. W. Ensink, R. Meier, P. Bauerfeind, U. Aufderhaar, S. Ketterer, K. Gyr and C. Beglinger</i>	370

Enhanced arterial intimal thickening after balloon catheter injury in diabetic animals accompanied by PDGF β -receptor overexpression of aortic media <i>T. Kanzaki, M. Shinomiya, S. Ueda, N. Morisaki, Y. Saito and S. Yoshida</i>	377	Commentary: Apolipoproteins and coronary heart disease <i>E. J. Schaefer</i>	441
Venous compliance and the venodilatory effect of nitroglycerin in insulin-dependent diabetic patients with and without (incipient) nephropathy <i>N. C. Schaper, A. J. H. M. Houben, Y. Schoon, J. P. Kooman, F. C. Huvers and A. C. Nieuwenhuijzen-Kruseman</i>	382	Lipoprotein Lp(a) as predictor of myocardial infarction in comparison to fibrinogen, LDL cholesterol and other risk factors: results from the prospective Göttingen Risk Incidence and Prevalence Study (GRIPS) <i>P. Cremer, D. Nagel, B. Labrot, H. Mann, R. Mucbe, H. Elster and D. Seidel</i>	444
The <i>in vivo</i> effect of interleukin-1 β on urea synthesis is mediated by glucocorticoids in rats <i>H. Heindorff, T. Almdal and H. Vilstrup</i>	388	Adhesion of <i>Helicobacter pylori</i> and <i>Escherichia coli</i> to human and bovine surface mucus cells <i>in vitro</i> <i>M. Nilius, G. Bode, M. Büchler and P. Malfertheiner</i>	454
β -adrenergic priming of rats <i>in vivo</i> modulates the effect of β -agonist <i>in vitro</i> on surfactant phospholipid metabolism of isolated lungs <i>W. Bernhard, B. Müller and P. Von Wichert</i>	393	Differing responses of cortisol to oCRF during endonasal and oral treatment with DDAVP <i>A. Carraro, M. Giusti, E. Porcella, P. Sessarego and G. Giordano</i>	459
Effects of lithium on bone resorption in cultured foetal rat long-bones <i>T. Papersack, J. Corvilain and P. Bergmann</i>	400	Increased serum angiotensin converting enzyme activity in type I insulin-dependent diabetes mellitus: its relation to metabolic control and diabetic complications <i>D. J. van Dyk, A. Erman, T. Erman, B. Chen-Gal, J. Sulkes and G. Boner</i>	463
Repression of ALA synthase by heme and zinc-mesoporphyrin in a chick embryo liver cell culture model of acute porphyria <i>S. M. Russo, J. A. Pepe, E. E. Cable, R. W. Lambrecht and H. L. Bonkovsky</i>	406	Post-prandial triglyceride-rich lipoprotein metabolism: possible role of sialylated apolipoprotein E isoproteins <i>H. Ito, C. Naito, Y. Suzuki, K. Nakamura and M. Nagase</i>	468
Plasma lipids in HIV-infected patients: a prospective study in 95 patients <i>J. Constans, J. L. Pellegrin, E. Peuchant, M. F. Dumon, I. Pellegrin, C. Sergeant, M. Simonoff, G. Brossard, P. Barbeau, H. Fleury, M. Clerc, B. Leng and C. Conri</i>	416	H ₂ -receptor antagonists are scavengers of oxygen radicals <i>D. Lapenna, S. de Gioia, A. Mezzetti, L. Grossi, D. Festi, L. Marzio and F. Cuccurullo</i>	476
Extended duration of vertical position might impair bone metabolism <i>S. A. Ben-Sasson, A. Finestone, M. Moskowicz, R. Maron, M. Weininger, I. Leichter, J. Margulies, P. Stein, M. Popovtzer, D. Rubinger, T. Rouash, H. Harrari and E. Galun</i>	421	Influence of digoxin-like immunoreactive factor on late complications in patients with diabetes mellitus <i>R. H. Straub, R. Elbracht, B. K. Krämer, M. Roth, K.-D. Palitzsch and J. Schölmerich</i>	482
Erythropoietic activity and iron metabolism in autologous blood donors during recombinant human erythropoietin therapy <i>D. H. Biesma, A. Van de Wiel, Y. Beguin, R. J. Kraaijenhagen and J. J. M. Marx</i>	426	Effects of selective LDL-apheresis and pravastatin therapy on platelet function in familial hypercholesterolaemia <i>A. Bröijersén, M. Eriksson, P. T. Larsson, O. Beck, L. Berglund, B. Angelin and P. Hjerdahl</i>	488
Number 7, July 1994		Rapid Communication: Induction of oxygen free radical generation in human monocytes by lipoprotein(a) <i>P. Riis Hansen, A. Kharazmi, M. Jauhiainen and C. Ehnholm</i>	498
Review: Positron emission tomography: applications to the investigation of movement disorders <i>E. D. Playford</i>	433		

Number 8, August 1994

- Review:** *Helicobacter pylori*
J. Calam 501
- Review:** Group A streptococcal antigens and superantigens in the pathogenesis of autoimmune arthritis
J. E. Taylor, D. A. Ross and J. A. Goodacre 511
- Commentary:** The roles of α -glucosidase inhibitors in diabetes
J. Donckier and G. Williams 522
- Commentary:** The lymphocyte Na^+/H^+ antiport and its activation by increased NaCl intake: the link with salt sensitivity and cellular Ca^{2+} regulation
A. Aviv 525
- The lymphocyte Na^+/H^+ antiport: activation in primary hypertension and during chronic NaCl-loading
B. O. Göbel, G. Hoffmann, M. Ruppert, K. O. Stumpe, H. Vetter, W. Siffert and R. Düsing 529
- Abdominal rubber drain piece aggravates intra-abdominal sepsis in the rat
W. Guo, V. Soltesz, Jin Wen Ding, R. Willén, Xiaoying Liu, R. Andersson and S. Bengmark 540
- Distribution of hepatitis B virus DNA sequences in different peripheral blood mononuclear cell subsets in HBs antigen-positive and negative patients
Y. Calmus, P. Marcellin, G. Beaurain, L. Chatenoud and C. Bréchet 548
- Inter-relations between the calcium set-points of Parfitt and Brown in primary hyperparathyroidism: a sequential citrate and calcium clamp study
P. Schwarz, H. A. Sørensen and I. Transbøl 553
- Cognitive brain function in non-demented patients with low-grade and high-grade carotid artery stenosis
C. Madl, G. Grimm, L. Kramer, R. Koppensteiner, M. Hirschl, W. Yeganehfar, M. M. Hirschl, A. Ugurluoglu, B. Schneider and H. Ehringer 559
- A 5 year controlled randomized study of prevention of postmenopausal trabecular bone loss with nasal salmon calcitonin and calcium
J. Y. Reginster, L. Meurmans, R. Deroisy, I. Jupsin, I. Biquet, A. Albert and P. Franchimont 565

Number 9, September 1994

- Review:** Mechanisms of exercise-induced asthma
H. K. Makker and S. T. Holgate 571
- Review:** Host defence capacities of pulmonary surfactant: evidence for 'non-surfactant' functions of the surfactant system
U. Pison, M. Max, A. Neuendank, S. Weißbach and S. Pietschmann 586
- Plasma homocysteine in relation to serum cobalamin and blood folate in a psychogeriatric population
K. Nilsson, L. Gustafson, R. Fäldt, A. Andersson and B. Hultberg 600
- Contribution of glycaemic control, endogenous lipoproteins and cholesteryl ester transfer protein to accelerated cholesteryl ester transfer in IDDM
M. C. Ritter and J. D. Bagdade 607
- Low levels of essential fatty acids are related to impaired delayed skin hypersensitivity in malnourished chronically ill elderly people
T. E. Cederholm, A. B. Berg, E. K. Johansson, K. H. Hellström and J. E. W. Palmblad 615
- Kinetics of circulating hyaluronan in humans
L. Lebel, J. Gabrielsson, T. C. Laurent and B. Gerdin 621
- Influence of age on cerebral potentials evoked by oesophageal balloon distension in humans
B. L. A. M. Weusten, H. G. Th. Lam, L. M. A. Akkermans, G. P. Van Berge-Henegouwen and A. J. P. M. Smout 627
- Urodilatin: a new approach for the treatment of therapy-resistant acute renal failure after liver transplantation
C. Cedidi, M. Meyer, E.-R. Kuse, P. Schulz-Knappe, B. Ringe, U. Frei, R. Pichlmayr and W.-G. Forssmann 632
- Number 10, October 1994**
- Review:** T cell recognition of hepatitis B and C viral antigens
M.-C. Jung, H. M. Diepolder and G. R. Pape 641
- Review:** Measuring progression of diabetic nephropathy
P. T. Sawicki and M. Berger 651
- Platelets in ulcerative colitis and Crohn's disease express functional interleukin-1 and interleukin-8 receptors
H. D. Schaufelberger, M. R. Uhr, C. McGuckin, R. P. H. Logan, J. J. Misiewicz, E. C. Gordon-Smith and C. Beglinger 656

Effect of 5-hydroxytryptamine antagonists on cholera toxin-induced secretion in the human jejunum <i>A. J. Eherer, T. A. Hinterleitner, W. Petritsch, U. Holzer-Petsche, E. Beubler and G. J. Krejs</i>	664	Hormone replacement therapy may reduce high serum homocysteine in postmenopausal women <i>M. J. van der Mooren, M. G. A. J. Wouters, H. J. Blom, L. A. Schellekens, T. K. A. B. Eskes and R. Rolland</i>	733
Postprandial apolipoprotein B100 and B48 metabolism in familial combined hyperlipidaemia before and after reduction of fasting plasma triglycerides <i>M. Castro Cabezas, D. W. Erkelens, L. A. W. Kock and T. W. A. De Bruin</i>	669	Platelet transmembrane signalling responses to collagen in familial hypercholesterolaemia <i>M. B. Cooper, K. C. B. Tan and D. J. Betteridge</i>	737
Enhancement of transforming growth factor β 1 expression in the rat pancreas during regeneration from caerulein-induced pancreatitis <i>T. Gress, F. Müller-Pillasch, H.-P. Elsässer, M. Bachem, C. Ferrara, H. Weidenbach, M. Lerch and G. Adler</i>	679	Effects of bile salt and phospholipid hydrophobicity on lithogenicity of human gallbladder bile <i>K. J. van Erpecum, P. Portincasa, M. F. J. Stolk, B. J. M. van de Heijning, E. S. van der Zaag, A. M. W. C. van den Broek, G. P. van Berge Henegouwen and W. Renooij</i>	744
Effect of experimental non-insulin requiring diabetes on myocardial microcirculation during ischaemia in dogs <i>L. Sebbag, R. Forrat, E. Canet, N. Wiernsperger, J. Delaye, S. Renaud and M. De Lorgeril</i>	686	Persistence of counter-regulatory abnormalities in insulin-dependent diabetes mellitus after pancreas transplantation <i>A. Battezzati, L. Luzi, G. Perseghin, E. Bianchi, D. Spotti, A. Secchi, S. Vergani, V. Di Carlo and G. Pozza</i>	751
Hepatic uptake and intestinal absorption of bile acids in the rabbit <i>R. Aldini, A. Roda, M. Montagnani, C. Polimeni, P. L. Lenzi, C. Cerre, G. Galletti and E. Roda</i>	691	The effect of probucol and vitamin E treatment on the oxidation of low-density lipoprotein and forearm vascular responses in humans <i>I. F. W. McDowell, G. M. Brennan, J. McEneny, I. S. Young, D. P. Nicholls, G. E. McVeigh, I. Bruce, E. R. Trimble and G. D. Johnston</i>	759
Diurnal variations in the plasma concentrations of mevalonic acid in patients with abetalipoproteinaemia <i>A. S. Pappu and D. R. Illingworth</i>	698	Inhibition of human vascular smooth muscle cell proliferation by lovastatin: the role of isoprenoid intermediates of cholesterol synthesis <i>E. Munro, M. Patel, P. Chan, L. Betteridge, G. Clunn, K. Gallagher, A. Hughes, M. Schachter, J. Wolfe and P. Sever</i>	766
Silicon and aluminium and their inter-relationship in serum and urine after renal transplantation <i>J. P. Bellia, K. Newton, A. Davenport, J. D. Birchall and N. B. Roberts</i>	703	Systemic interleukin-1 α and interleukin-2 secretion in response to acute stress and to corticotropin-releasing hormone in humans <i>H. M. Schulte, C. M. Bamberger, H. Elsen, G. Herrmann, A. M. Bamberger and J. Barth</i>	773
Erratum	711	Rapid Communication: Influence of weight reduction on platelet volume: different effects of a hypocaloric diet and a very low calorie diet <i>H. Toplak and T. C. Wascher</i>	778
Referees for 1993	712	Rapid Communication: Hepatitis C virus infection in non-Hodgkin's B-cell lymphoma complicating mixed cryoglobulinaemia <i>C. Ferri, M. Monti, L. La Civita, G. Careccia, C. Mazzaro, G. Longombardo, F. Lombardini, F. Greco, G. Pasero, S. Bombardieri and A. L. Zignego</i>	781
Number 11, November 1994			
Review: Apoptosis in disease <i>J. Savill</i>	715		
The HELP-LDL-apheresis multicentre study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. II. Final evaluation of the effect of regular treatment on LDL-cholesterol plasma concentrations and the course of coronary heart disease <i>P. Schuff-Werner, H. Gohlke, U. Bartmann, G. Baggio, M. C. Corti, A. Dinsenhacher, T. Eisenhauer, P. Grützmaker, C. Keller, U. Kettner, W. Kleophas, W. Köster, C. J. Olbricht, W. O. Richter, D. Seidel and the HELP-Study Group</i>	724		

Number 12, December 1994

Review: Hypoglycaemia unawareness in insulin-dependent diabetes mellitus <i>Th. F. Veneman and T. W. van Haefen</i>	785	Time course of haemodynamic changes after maximal exercise <i>J. E. Isea, M. Piepoli, S. Adampoulos, G. Pannarale, P. Sleight and A. J. S. Coats</i>	824
Erythrocyte Na ⁺ -H ⁺ exchanger and Na ⁺ -Li ⁺ countertransport activity in primary aldosteronism <i>P. Delva, C. Pastori, M. Degan, G. Montesi, A. Bassi and A. Lechi</i>	794	A study of factors governing fluid filtration in the diabetic foot <i>G. Rayman, S. A. Williams, J. Gamble and J. E. Tooke</i>	830
Increase of tumour necrosis factor α synthesis and gene expression in peripheral blood mononuclear cells of children with idiopathic nephrotic syndrome <i>C. Bustos, E. González, R. Muley, J. L. Alonso and J. Egido</i>	799	Influence of lung volume on collateral resistance during methacholine-induced bronchospasm <i>M.-C. Coté, F. Sériès, M. Laviolette, F. Laberge, L. Atton and Y. Cormier</i>	837
Serum ionized calcium, intact PTH and novel markers of bone turnover in bedridden elderly patients <i>A. Sorva, M. Välimäki, J. Risteli, L. Risteli, S. Elfving, H. Takkunen and R. Tilvis</i>	806	Minimal model analyses of insulin sensitivity and glucose-dependent glucose disposal in black and white Americans: a study of persons at risk for type 2 diabetes <i>K. Osei and D. A. Cottrell</i>	843
Effect of degenerative spinal and aortic calcification on bone density measurements in post-menopausal women: links between osteoporosis and cardiovascular disease? <i>L. M. Banks, B. Lees, J. E. MacSweeney and J. C. Stevenson</i>	813	Rapid Communication: The pathophysiology of the insulin-like growth factor axis in fetal growth failure: a basis for programming by undernutrition? <i>K. Langford, W. Blum, K. Nicolaidis, J. Jones, A. McGregor and J. Miell</i>	851
Kinetics of ¹³ CO ₂ elimination after ingestion of ¹³ C bicarbonate: the effects of exercise and acid base balance <i>G. P. Leese, A. E. Nicoll, M. Varnier, J. Thompson, C. M. Scrimgeour and M. J. Rennie</i>	818	Author Index	857
		Keyword Index	861

Local serum application: restoration of sufficient host defense in human peritonitis

A. G. BILLING*, D. FRÖHLICH*, G. KONECNY*, F. W. SCHILDBERG*, W. MACHLEIDT†, H. FRITZ‡ & M. JOCHUM‡ *Chirurgische Klinik und Poliklinik der Universität München, Klinikum Großhadern, Marchioninstraße 15, D-81377 München; †Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Goethestr., D-80336 München and ‡Abteilung für Klinische Chemie und Klinische Biochemie in der Chirurgischen Klinik Innenstadt der Universität München, Nußbaumstraße 20, D-80336 München, Germany

Received 15 February 1993 and in revised form 21 June 1993; accepted 29 June 1993

Abstract. Intra-abdominal host defense in human peritonitis is hampered by a severe dysfunction of phagocytosis due to an almost complete breakdown of bacteria opsonization. This defect relates to some opsonin consumption, but mainly to proteolytic and oxidative opsonin destruction. To restore and protect intact opsonins we have developed a clinical approach of intra-operative peritoneal serum application. In a prospective, controlled, and randomized study of 30 patients with generalized peritonitis we have investigated the impact of this adjuvant therapy on biochemical parameters and clinical features. Serum application induced a rise in opsonin concentration and, even more pronounced, opsonin function ($P < 0.01$) of several hours' duration, leading to a distinct improvement of bacteria elimination. In addition, α_1 -proteinase inhibitor (α_1 -PI) levels were significantly increased after 1 h ($P < 0.05$) in the treatment group.

The follow-up by APACHE II scoring indicated an improvement in the therapy group over the whole observation period of 14 days. Lethality in the therapy group was 33% compared to 53% in controls. These results indicate that the intra-operative restoration of physiologic intra-abdominal milieu can improve bacteria opsonization and elimination, thus contributing to a favourable clinical course in abdominal sepsis.

Keywords. Host defense, opsonins, opsonization, peritonitis, proteolysis.

Introduction

In treating abdominal sepsis the surgical eradication of the source of peritonitis is the crucial prerequisite for a favourable outcome [1]. At the end of the surgical procedure, however, large quantities of bacteria remain in the abdominal cavity in spite of thorough

mechanical cleansing and lavage [2]. After therapeutic abdominal lavage procedures some part of the rinse solution is inevitably left behind. Such fluid has been proven to further hamper bacterial elimination probably because it dilutes the local defense components [3]. Thus, in the postoperative course the result of the competition between bacterial growth and bacteria elimination by physiological defense systems and therapeutic manoeuvres determines the patient's situation. Persisting abdominal infection in this situation leads to immunosuppression, endotoxaemia and septic organ failure causing a high mortality [4,5]. Just recently, we could demonstrate pronounced pathological changes of the local intra-abdominal setting in abdominal infection resulting in a severe impairment of the local defense capacity [6]. Large quantities of neutrophils undergo extensive stimulation by complement split products, cytokines and bacteria with a consecutive drastic release of both lysosomal proteinases and oxygen metabolites. In purulent peritonitis exudates proteinase inhibitor consumption became obvious allowing proteolytic as well as oxidative destruction of functional proteins, e.g. proteinase inhibitors, complement components and immunoglobulins. Thus bacteria opsonization by complement and immunoglobulin components, a main prerequisite for sufficient phagocytosis, was almost abolished in such exudates, although the numerous phagocytes were highly prestimulated and intact regarding particle uptake and metabolic activities [6,7,8]. In order to restore a physiologic assembly of the intra-abdominal humoral defense components three main aspects have to be taken into account:

- 1 Supplementation of intact opsonins (complement components and immunoglobulins).
- 2 Application of proteinase inhibitors to prevent further proteolytic degradation.
- 3 Supply of radical scavengers or oxidizable substrates to detoxify oxygen metabolites and thus protect functional proteins.

Correspondence: Priv. Doz. Dr A. G. Billing, Chirurgische Klinik und Poliklinik der Ludwig-Maximilians-Universität München, Klinikum Großhadern, Postfach 70 12 60, D-W 81377 München, Germany.

Table 1. The Mannheim Peritonitis Index

Risk factors	Loading (if present)
Age over 50 years	5
Female sex	5
Organ failure	7
Non-related malignancy	4
Pre-operative peritonitis (> 24 h)	4
Primary focus not colon	4
Diffuse generalized peritonitis	6
Exudate	
clear	0
cloudy-purulent	6
fecal	12

To meet all these requirements and coincidentally develop an approach ready for clinical application we chose the local intra-abdominal application of normal serum. Thereby the ideally balanced composition of humoral defense systems, as developed by evolution itself, was implanted in the abdominal environment. The impact of this therapeutic approach for human abdominal sepsis on the efficiency of local host defense parameters was evaluated.

Patients and methods

Thirty consecutive patients with abdominal sepsis were enrolled into this prospective, controlled, and randomized study. The study protocol had been approved by the local ethical commission. Entrance criteria were: diffuse peritonitis (purulent exudate in more than one quadrant); leukocyte count in exudate $> 3000 \mu\text{l}^{-1}$; surgical eradication of the source of peritonitis possible (e.g. not pancreatitis); no primary peritonitis (infected ascites); age over 18 years. After intra-operative diagnosis of a diffuse peritonitis, patients qualifying for the study on basis of the mentioned criteria were randomized into two groups. Surgical treatment followed standard procedures [9] including identical antibiotic regimens. In patients on risk for persistent peritonitis scheduled relaparotomies were performed every other day until sanitation of the abdominal cavity was obtained. In all cases an intra-operative abdominal lavage was performed with 10 l of Ringer's solution. In the therapy group one unit (about 300 ml) of blood-group compatible normal donor serum (fresh frozen serum supplied by the blood bank) was then applied intra-abdominally to the patient. Testing and safety procedures for serum were the same as for fresh frozen plasma. Serum application was preferred (instead plasma) to avoid fibrin deposition. In control patients no sham therapy was added, because intra-abdominal application of inert fluids is known to deteriorate bacterial elimination [3].

Patient classification

By the time of the first operation scoring of severity was performed with the Mannheim Peritonitis Index [10,11] (see Table 1) and the APACHE II system

[12,13,14]. A follow-up was performed with the APACHE II on days 3, 7, 10 and 14, as suggested by the Surgical Infection Society [15].

Sampling procedures

Abdominal exudate was drawn at the beginning and the end of the peritonitis operation and drainage effluate was pooled at 1, 2 and 8 h postoperatively. These time intervals were chosen according to pilot studies to best follow the opsonin kinetics. Blood samples were drawn simultaneously and processed to serum and EDTA-plasma. Aliquots of the exudates were centrifugated at $2000 \times g$ for 10 min and stored at -70 C .

Microbiology

Exudate samples were incubated in aerobe and anaerobe media (culture bottles Bactec, Becton Dickinson). At the same time culture plates (blood agar and McConkey agar) were inoculated with crude exudate and exudate dilution 1:100. Processing and analysis followed microbiological standard procedures. Results are given as colony forming units (CFU) and relative CFU, that is CFU in percent of initial concentration.

Protein concentration

To allow for precise determination of the low protein concentrations in postoperative exudates a sensitive assay (Protein Micro Determination, Sigma) was employed according to the manufacturer's instruction. The serum standard was $7.1 \text{ g } 100 \text{ ml}^{-1}$.

Concentration of opsonins IgG and complement C3, and of α_1 -proteinase inhibitor (α_1 PI)

Immunological quantification of all three proteins was performed by radial immunodiffusion on standardized (NOR) plates (Behringwerke, Marburg, Germany). For exact measurement a reading projector was employed. C3 plates contained C3c antibody thus measuring whole C3 and C3c containing fragments. To prevent complement activation all tests were performed in EDTA-exudates. The plasma standard was $79.9 \text{ mg } 100 \text{ ml}^{-1}$. IgG plates contained polyclonal antihuman IgG Gamma antibody. The serum standard was 11.3 g l^{-1} . For the α_1 PI concentration the serum standard was $229 \text{ mg } 100 \text{ ml}^{-1}$.

α_1 PI activity

Alpha₁ PI function was determined as trypsin inhibition employing a specific chromogenic substrate for trypsin (α_1 antitrypsin test kit, Boehringer Mannheim).

Chemiluminescence assay for opsonic activity

Opsonic activity was determined using a specific modification of a chemiluminescence assay [16].

Table 2. Individual patient classification

No.	Age	Sex	Peritonitis		Operation	Primary surg.succ.	Survival
			origin	exudate			
A serum application (therapy group)							
1	87	f	perf.ulcer	fi	simple suture	y	y
2	76	f	perf.appendix	fi	appendectomy	y	y
3	78	f	sigma ca	fe	Hartmann proc.	n	n
4	88	m	polypectomy	b	simple suture	y	n
5	63	f	gastric lymphoma	fi	gastrectomy	y	n
6	81	f	gastric perf.	p	simple suture	y	y
7	78	m	gastric perf.	fi	B l-resection	y	y
8	78	f	gastric perf.	fi	simple suture	y	y
9	39	f	endometriosis colonperf.	fe	Hartmann proc.	y	y
10	59	m	colonperf.	fe	resection	n	n
11	50	f	perf.ulcer	fi/p	B l-resection	n	n
12	89	m	rectum-ca	p/fe	Hartmann proc.	y	n
13	83	f	sigma ca	p	resection	n	n
14	48	f	Meckel's div.	p	ileum resection	n	n
15	69	f	diverticulitis	fe	Hartmann proc.	y	y
B no serum application (control group)							
1	74	m	post gastrectomy	fi/b	suture, drain.	n	n
2	69	f	colon fistula	fi/p	a.p., excision	n	n
3	84	f	colon necrosis	fi	resection	y	y
4	56	f	intest.leakage	p	suture	n	y
5	93	f	sigmaperf.	fe	resection	y	n
6	61	f	intest.leakage	fi/p	resection	y	y
7	75	f	mesenteric ischaemia	fi/p	resection	y	y
8	71	f	colonperf.	p	Hartmann proc.	y	y
9	69	m	colon ca	p	resection	y	y
10	74	f	Hartmann necrosis	p	resection	y	y
11	66	m	colon ca	fe	resection	n	n
12	75	f	gastric perf.	p	suture	n	n
13	61	f	colonperf.	fe	Hartmann proc.	y	y
14	64	f	diverticulitis	fe	Hartmann proc.	y	y
15	51	m	gastric perf.	p	suture	y	y

fe = fecal; fi = fibrinous; b = bile; p = purulent; primary surg.succ. = successful surgical sanitation on first operation; y = yes; n = no.

Briefly, zymosan A was pre-opsonized by incubation with normal serum, patient's serum or patient's exudate for 15 min at 37 °C respectively. The final assay solution contained 0.05 ml diluted EDTA blood from healthy donors (1:15 in phosphate-buffered saline (PBS)), 0.8 ml Veronal buffer and 0.1 ml luminol solution (0.7 mM), resulting in a final blood dilution of 1:300. The reaction was started by adding 0.05 ml of pre-opsonized zymosan (20 mg ml⁻¹). Chemiluminescence was measured with the six channel Biolumat LB9505 (Laboratorium Professor Berthold, Wildbad, Germany) and the results were integrated over 30 min by means of a microcomputer (Apple IIe). Opsonic activity of patient serum or exudate was expressed as the percentage of the value obtained using normal serum (= 100%) [16].

Statistics

Statistical analysis was performed with SPSS software. Figures display mean and (double) SEM. Group comparison for continuous variables employed the Wilcoxon test for related and the Mann Whitney test

for unrelated variables. Classified parameters were compared by Chi square tests.

Results

Patient characteristics

Individual patient classification is listed in Table 2 as to cause and characteristics of peritonitis, surgical procedure, success of surgical cure within one operation, and outcome. The two groups were comparable as to age, sex ratio and the underlying cause of peritonitis (see Table 3). The therapy group revealed a trend towards a higher rate of malignoma and postoperative peritonitis. Due to technical reasons operation time varied from 0.5 to 3 h. Generally it was short in simple suture and lavage operations but longer in anatomical resection.

Scoring the severity of peritonitis during the first operation with the Mannheim Peritonitis Index and APACHE II both systems assigned the therapy group to higher scores, indicating increased risk (see Table 3). The follow-up by APACHE II revealed considerable differences between both groups especially within the

Table 3. Group classification (MPI = Mannheim Peritonitisindex: median:range)

	Therapy group (n = 15)	Controls (n = 15)
Male:female	1:2.0	1:2.8
Age (years)	67.5/42	70.1/50
APACHE II	17/18	16/11
MPI	27.1/22	25.4/17
Malignancy (%)	53.3	33.3
Postoperative peritonitis (%)	40.0	33.3

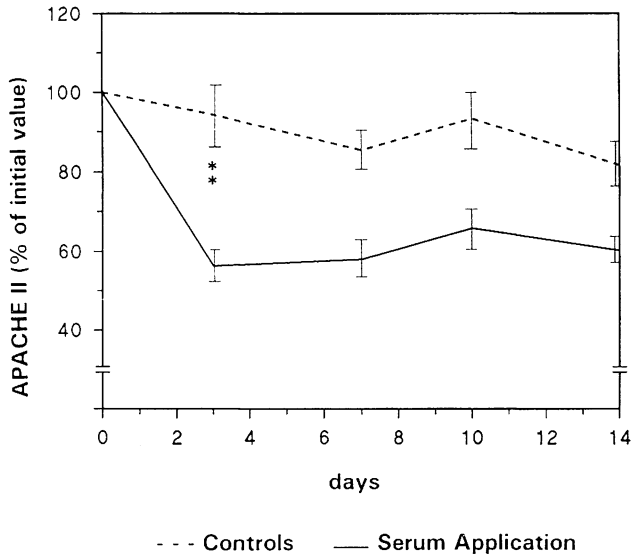


Figure 1. Follow-up of the APACHE II score on postoperative days 3, 7, 10 and 14 (in percent of initial value = 100%; difference significant with ** $P < 0.01$).

Table 4. Classification of the clinical course (median range)

	Therapy group (n = 15)	Controls (n = 15)
Re-operations required pat.	2.5/13	3.9/11
ICU treatment (days)	20.5/53	24.1/88
Hospital stay (days)	27.3/88	29.3/88
Survival (%)	66.7	46.7

first days. In controls APACHE was fairly constant with (median/range) 16/11 intra-operatively, 15/16 on day 3 and 14/11 on day 14. In the therapy group APACHE (median/range) was 17/18 initially, dropped to 10/20 on day 3 and was 12/15 on day 14. In Fig. 1 the course of APACHE II is delineated related to the initial value (= 100%); on day 3 the difference between both groups was significant ($P < 0.01$).

Therapy group patients required less reoperations for complete sanitation (mean values 2.0 versus 3.4) (see Table 4). The ICU stay and total hospital stay

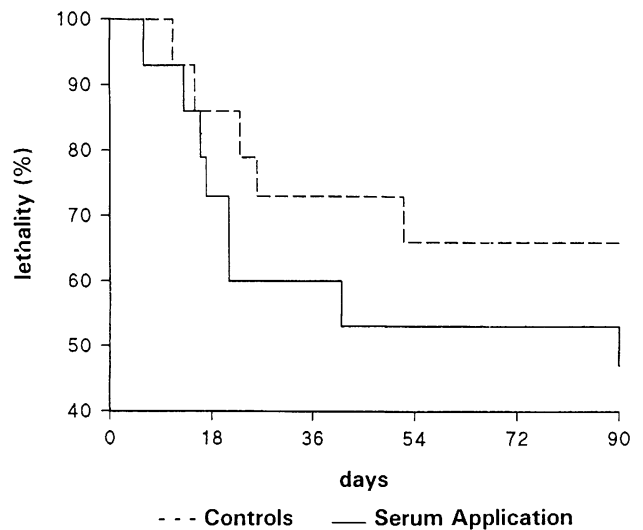


Figure 2. Survival rate according to Kaplan-Meier for peritonitis patients with and without serum application.

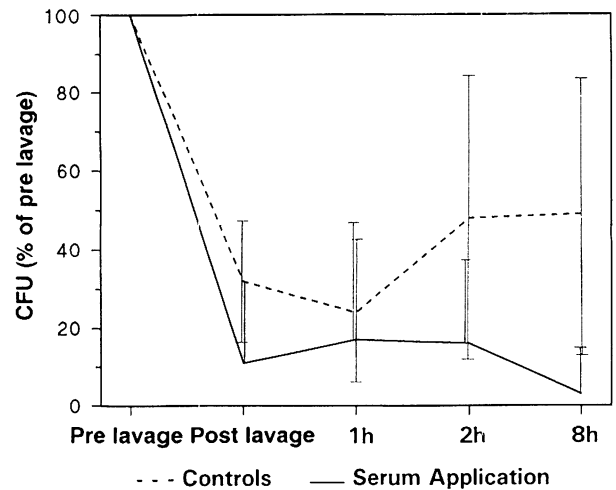


Figure 3. Impact of the serum application on colony forming units (CFU) in peritoneal exudate (% of initial exudate concentration = 100%).

were similar in both groups. All time intervals date from the onset of peritonitis. Lethality in the study group was 33% compared to 53% in controls. Due to the sample size this difference was not significant in a log rank test. The difference in survival became obvious only 2-3 weeks after operation (see Fig. 2).

Microbiological findings

The number of colony forming units (CFU) in the exudates varied considerably. The extensive abdominal lavage led only to a moderate bacteria elimination. This bacteria concentration remained rather constant in controls. Therapy group exudates revealed a slight increase after 1 h, yet, after 8 h a considerably reduced bacteria concentration was found in this group. In Fig. 3

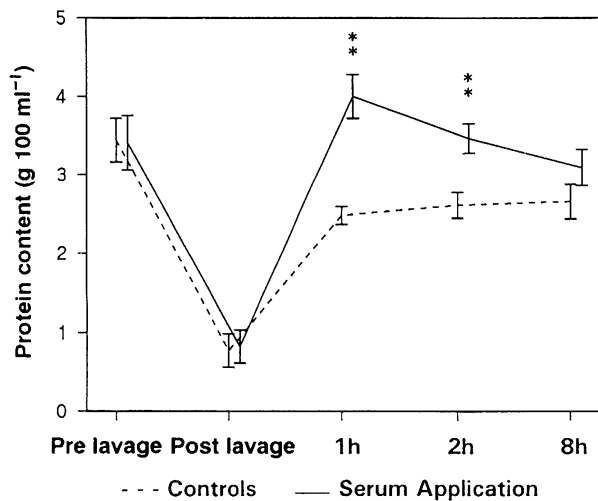


Figure 4. Impact of the serum application on the overall protein content (g dl^{-1}) in peritoneal exudate. Significant difference with $**P < 0.01$.

CFU concentrations are delined in relation to the initial value (= 100%). After 8 h CFU concentrations were 49% of initial in controls but only 3% of initial in serum treated patients. *E. coli* was predominant, anaerobe bacteria were present in 10% of the patients.

Protein content

The median serum protein content of the control group (median/range) was $4.3/2.2 \text{ g dl}^{-1}$ and of the therapy group $4.0/2.4 \text{ g dl}^{-1}$. In the peritonitis exudate of the control group the concentration was $4.1/3.8 \text{ g dl}^{-1}$, in the therapy group $3.4/4.5 \text{ g dl}^{-1}$. In both groups protein content was reduced to a similar extent by the abdominal lavage (see Fig. 4). Serum application resulted in a pronounced increase of protein concentration after 1 and 2 h, respectively. This increase was significantly higher than in the control group ($P < 0.01$). Eight hours post lavage the protein content in both groups was approximately the same.

Complement C3

Entrance median C3 serum concentration (median/range: $52.4/24.9 \text{ mg dl}^{-1}$) and C3-peritonitis exudate concentration of the control group ($49.2/74.2 \text{ mg dl}^{-1}$) exceeded insignificantly the corresponding therapy group concentrations (C3 in serum $51.9/27.7 \text{ g dl}^{-1}$; C3 in peritonitis exudate: $33.3/49.5 \text{ mg dl}^{-1}$) (see Fig. 5). Lavage reduced the intraperitoneal C3-concentration both in controls and in the therapy group. The postoperative C3-concentration in drainage effluat in controls raised up to (median/range) $21.8/32.7 \text{ mg dl}^{-1}$ after 1 h and remained rather stable in the further course. In contrast, local serum application led to a drastic increase of C3-concentration after 1 h followed by a moderate decrease after 2 h and after 8 h, respectively. The group differences were significant up to 2 h ($P < 0.05$) post-treatment.

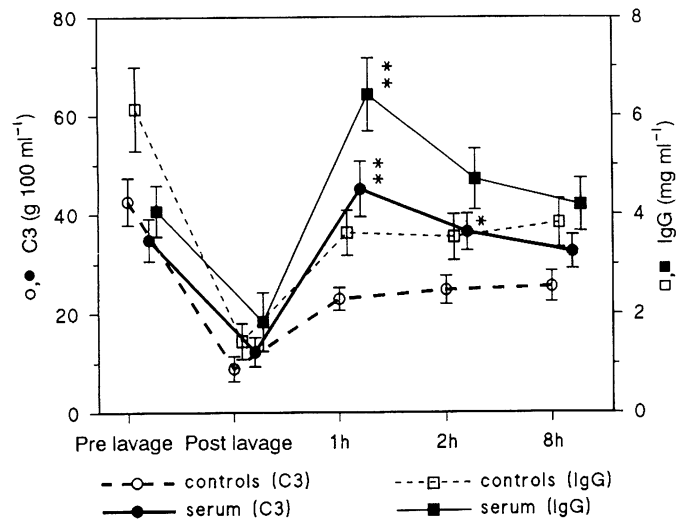


Figure 5. Impact of the serum application on opsonin concentration in peritoneal exudates (C3: —○—, IgG: —■—). Significant difference with $**P < 0.01$; $*P < 0.05$.

Immunoglobulin G

IgG serum concentrations were subnormal in all study patients. In the control group initial IgG serum concentration (median/range: $6.6/3.9 \text{ g l}^{-1}$) as well as IgG peritonitis exudate concentration ($5.6/10.7 \text{ g l}^{-1}$) exceeded the corresponding concentrations found in the therapy group (IgG in serum $5.4/4.1 \text{ g dl}^{-1}$; IgG in peritonitis exudate $4.2/10.2 \text{ g l}^{-1}$). The abdominal lavage reduced IgG concentration both in controls and in the therapy group (see Fig. 5). In controls IgG concentration was moderately increased after 1 h and then remained rather constant. Local serum application, however, led to an immediate rise of the IgG concentration up to (median/range) $6.2/7.2 \text{ g l}^{-1}$ after 1 h. After 2 h the concentration was reduced to $5.1/8.4 \text{ g l}^{-1}$ and after 8 h to $4.2/8.2 \text{ g l}^{-1}$. One hour after treatment a significant ($P < 0.01$) difference between both patient groups became obvious.

Opsonic capacity

The median opsonic capacity in serum was slightly subnormal, both in control (median/range: $75.5/38\%$) and in therapy patients ($76.6/48\%$). In peritonitis exudates opsonic capacity in controls ($6.0/27\%$) was quite different from the activity in therapy-group exudates ($14/50\%$) (see Fig. 6). The intra-operative lavage drastically reduced these values. In controls a moderate postoperative recovery was observed. In contrast, serum application led to a pronounced increase of intra-abdominal opsonic capacity after 1 h, which persisted over the whole observation period. The differences between both groups were highly significant ($P < 0.01$) throughout this post-treatment period.

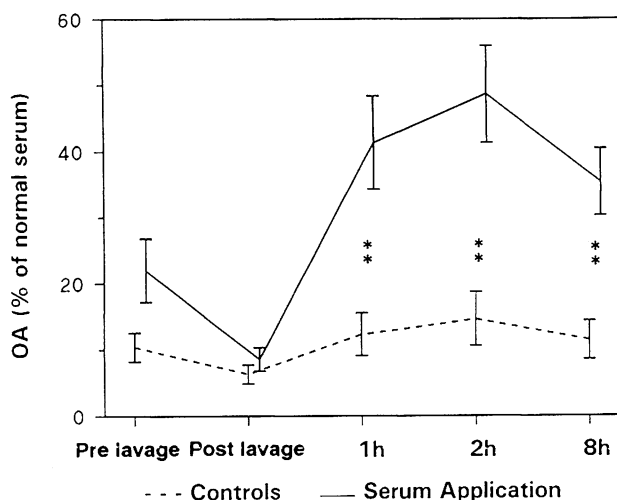


Figure 6. Impact of serum application on the opsonin activity in peritoneal exudates (OA, in percent of normal serum activity). Significant difference with $**P < 0.01$.

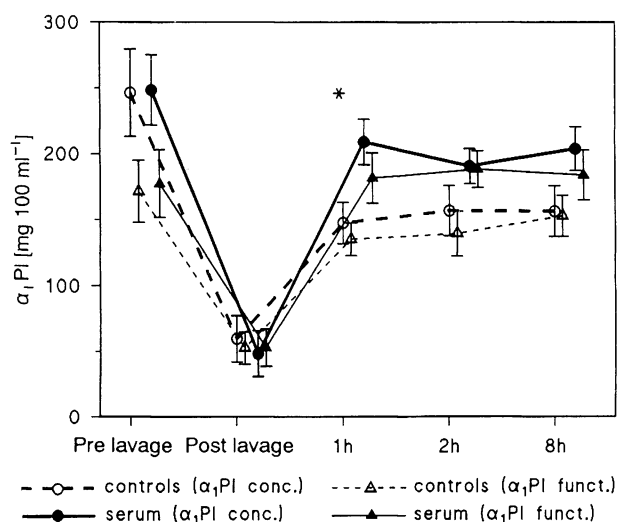


Figure 7. Impact of serum application on the α_1 -PI concentration ($\text{---}\circ\text{---}$) and α_1 -PI function ($\text{---}\blacksquare\text{---}$) in peritoneal exudate. Significant difference with $*P < 0.05$.

α_1 -Proteinase inhibitor

Median serum concentrations of α_1 PI were enhanced to the upper limit of normal in all study patients (controls: (median/range) 243/198 mg dl⁻¹; therapy group: 279/172 mg dl⁻¹). In the exudates both groups revealed a similar peri-operative pattern (260/449 mg dl⁻¹ in controls and 283/345 mg dl⁻¹ in the therapy group) (see Fig. 7). By the end of the operation the concentration declined. The serum application provoked a distinct rise of the α_1 PI concentration whereas in controls only a moderate increase was noticed. In the further course controls and therapy group showed parallel though not identical concentration patterns with significant differences only 1 h after serum

application ($P < 0.05$). α_1 PI function in peritonitis exudates revealed a similar course, but about 30% of the immunologically detected α_1 PI was inactive in both patient groups (see Fig. 7).

Discussion

The first hours and days after operative treatment of peritonitis essentially determine the further course of the disease. Increase of oxygenation and haemodynamical stabilization indicate a favourable development, progressing multiorgan dysfunction characterizes a persistence of septic problems. A definite failure to eliminate the bacteria from the abdominal cavity leads to abscess formation or recurrent abdominal sepsis. Even a delay in bacteria reduction is accompanied by prolonged endotoxaemia, leading to immunosuppression with high risk for the development of septic organ failure [4,5,17]. A specific clinical follow-up of intra-abdominal inflammation and infection in the early peri-postoperative hours of peritonitis has not been presented so far. Thus, our data provide for the first time detailed information about the impact of the operative treatment on microbiological and biochemical parameters involved in this disease and substantiate a novel approach in treating peritonitis patients.

Postoperative course without serum

Our data give striking evidence of the great amount of bacteria remaining in the peritoneal cavity at the end of a presumably effective operation. Similar results from animal studies have been published by Edmiston *et al.* [2] indicating that a major part of bacteria adhere to mesothel and fibrin. Yet, systematical quantitative bacteriological controls of the intra-operative lavage procedure have not been published up to now in the relevant literature [18].

According to our results the lavage procedure lead to a more or less pronounced reduction of all proteins. The rather wide variation is probably due to the interval between lavage and the end of the operation (when the 'post lavage samples' were drawn), rather than to a different efficacy of the lavage itself.

Although in the 'spontaneous' course (without serum application) a fast, moderate influx of serum proteins like IgG and C3 could be observed, the increase of activity of the opsonins IgG and C3 was very limited. The distinct divergence between opsonin concentration and function persisted, indicating rapid inactivation of the circulation-derived proteins in the peritoneal cavity.

Impact of the local serum application on the intra-abdominal infection

The local application of normal serum abruptly, though short-termed, created a 'physiological milieu' in the abdominal cavity, providing the treated patients with a complete and intact humoral and antiproteoly-

tic defense system. But obviously the applied serum underwent substantial diminution by means of resorption and exudation already during the first hours after intraperitoneal administration.

We could demonstrate a trend towards an improvement of bacteria elimination in the therapy group, which became especially obvious after 8 h. At this time a vigorous influx of granulocytes from the circulation occurs [19,20], bringing about abundant phagocytic capacity. Thus the conclusion seems reasonable that the intra-abdominal serum application provoked a regeneration of phagocytosis by a restitution of bacteria opsonization. Interestingly, the substitution of functionally intact proteins was more striking than the mere rise of the protein concentration. Compared to the controls, therapy group opsonins revealed a three-times increased function/concentration ratio, indicating that the high content of antioxidants [21] and proteinase inhibitors [22] in the administered serum protected the opsonins from oxidative and/or proteolytic inactivation. In this respect, the restoration of α_1 PI levels in the peritoneal cavity due to local serum instillation may serve also as an indicator for the supplementation of other important proteinase inhibitors (e.g. α_2 -macroglobulin).

Impact of the local serum application on the clinical course

Abdominal sepsis can develop from a considerable variety of underlying diseases and thus presents as a inhomogenous and polymorph syndrome. Therefore, the impact of single therapeutic additives on the clinical course is difficult to evaluate [8,15,23]. Besides the normalization of biochemical parameters any new therapeutic approach aims at improvement of the clinical course. Such benefit, however, is hard to define. Most peritonitis and sepsis scores are not constructed for follow-up purposes [24,25]. Just recently, the complexity of peritonitis studies has been discussed in extense [15]. After thorough evaluation, scoring with the APACHE II system on days 0, 3, 7, 10 and 14 has been recommended by this group of specialists, even though some of the APACHE II parameters are subject to easy therapeutic correction and the index has originally been designed for an initial graduation only [12,13,14,26]. In this respect some modifications to improve continuous APACHE II-scoring have been proposed [27].

Compared to controls the APACHE II score in our therapy group revealed a slightly higher initial (pre-operative) infection severity but a considerably more favourable further course in these patients. Persistence or increase of septic organ failure in the course of peritonitis therapy is associated with persisting or recurrent abdominal sepsis and it carries a poor prognosis [4,5]. The APACHE II difference between our two study groups as a parameter of sepsis and organ failure was especially pronounced and significant on day 3 after serum application, indicating a

positive early impact of this therapy on relevant pathophysiologic features.

Both study groups were well comparable as to age, sex and underlying diseases. The higher initial APACHE II score and higher incidence of underlying malignoma in the study group indicated a higher severity of disease. Yet, these patients required slightly shorter ICU treatment and hospitalization. Lethality in the study group was 20% lower than that of controls. This difference marks a distinct positive trend, yet, due to the sample size, statistical significance is not achieved.

Lethality in abdominal sepsis is mainly caused by septic organ failure. Such organ impairment develops in the early stage or in recurrent sepsis due to inflammatory mediators (e.g. endotoxin, TNF, activated complement components) but can be compensated for a long time by means of modern intensive care treatment (e.g. technical organ substitution by ventilation, haemofiltration etc). This phenomenon is reflected by our results. The positive impact of serum application on the (sepsis and organ failure related) APACHE II score was especially pronounced in the early stage, whereas the difference in lethality became obvious only after 3 weeks.

Unvariably, the urgent and definite surgical cure of the source of peritonitis is of central importance. Our results indicate, however, that an adjuvant therapy, aligned to pathophysiological mechanisms, might essentially contribute to a reduction of the still high lethality of abdominal sepsis. Our new therapeutic approach, that is local serum application, turned out to successfully ameliorate the severe impairment of bacteria opsonization that had previously been demonstrated in detail in peritonitis exudate by our group [6]. Although in the present study a beneficial effect of local serum could be demonstrated for the crucial initial time after operation, a prolonged therapy by repeated application should be of even more beneficial efficiency and thus deserves further investigation.

Acknowledgment

The technical assistance of Mrs R. Hell and Mrs M. Meier is gratefully appreciated. Major parts of the work was financially supported by the Sonderforschungsbereich 207 of the University of Munich (grants G1 to A.B. and W.M., G5 to M.J.). Parts of the results are from G. Konecny's thesis.

References

- 1 Billing A, Fröhlich D, Mialkowskyj O, Stokstad P, Schildberg FW. Peritonitisbehandlung mit der Etappenlavage: Prognosekriterien und Behandlungsverlauf. *Langenbecks Arch Chir* 1992;377:305-13.
- 2 Edmiston CE, Goheen MP, Kornhall S, Jones FE, Condon RE. Fecal peritonitis: microbial adherence to serosal mesothelium and resistance to peritoneal lavage. *World J Surg* 1990;14:176-83.
- 3 Dunn DL, Barke RA, Ahrenholz DH, Humphrey EW, Simmons

- RL. The adjuvant effect of peritoneal fluid in experimental peritonitis. *Ann Surg* 1984;199:1:37-43.
- 4 Maddaus MA, Ahrenholz D, Simmons RL. The biology of peritonitis and implications for treatment. *Surg Clin N Am* 1988;68:2:431-3.
 - 5 Rotstein OD, Meakins JL. Diagnostic and therapeutic challenges of intraabdominal infections. *World J Surg* 1990;14:159-66.
 - 6 Billing A, Fröhlich D, Kortmann H, Jochum M. Die Insuffizienz der intraabdominellen Infektabwehr bei der eitrigen Peritonitis—Folge einer gestörten Fremdkörperopsonierung. *Klin Wochenschr* 1989;67:349-56.
 - 7 Dunn DL, Barke RA, Knight NB. Role of resident macrophages, peritoneal neutrophils, and translymphatic absorption in bacterial clearance from the peritoneal cavity. *Inf Immun* 1985;49:257-64.
 - 8 Freischlag J, Backstrom B, Kelly D, Keehn G, Busutill BA. Comparison of blood and peritoneal neutrophil activity in rabbits with and without peritonitis. *J Surg Res* 1986;40:145-51.
 - 9 Günther B, Billing A, Heberer G. Grundzüge der chirurgischen Behandlung der Peritonitis. *Anästh Intensivmed* 1987;28:141-8.
 - 10 Linder MM, Wacha H, Feldmann U, Wesch G, Steifensand RA, Gundlach E. Der Mannheimer Peritonitisindex. Ein Instrument zur intraoperativen Prognose der Peritonitis. *Chirurg* 1987;58:84-92.
 - 11 Wacha H, Linder MM, Feldmann U. Mannheim peritonitis index—prediction of risk of death from peritonitis: construction of a statistical and validation of an empirically based index. *Theor Surg* 1987;1:169-77.
 - 12 Bohnen JMA, Mustard RA, Oxholm SE, Schouten D. Apache II score and abdominal sepsis. *Arch Surg* 1988;123:225-9.
 - 13 Knaus WA, Draper EA, Wagner DP, Zimmerman JE. Apache II: a severity of disease classification system. *Crit Care Med* 1985;13:818-29.
 - 14 Knaus WA, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE—acute physiology and chronic health evaluation: a physiologically based classification system. *Crit Care Med* 1981;9:8:591-7.
 - 15 Nyström PO, Bax R, Dellinger EP *et al.* Proposed definitions for diagnosis, severity scoring, stratification, and outcome for trials on intraabdominal infections. *World J Surg* 1990;14:148-58.
 - 16 Billing A, Fröhlich D, Jochum M, Kortmann H. Impaired phagocytosis in peritonitis secondary to complement consumption. *Surg Res Comm* 1988;3:335-45.
 - 17 Christou NV. Systemic and peritoneal host defense in peritonitis. *World J Surg* 1990;14:184-90.
 - 18 Schein M, Saadia R, Decker G. Intraoperative peritoneal lavage. *Surg Gynecol Obstet* 1988;166:187-95.
 - 19 Dunn DL, Barke RA, Ewald DC, Simmons RL. Macrophages and translymphatic absorption represent the first line of host defence of the peritoneal cavity. *Arch Surg* 1987;122:105-10.
 - 20 Skau T, Nyström PO, Öhman L, Stendahl O. Bacterial clearance and granulocyte response in experimental peritonitis. *J Surg Res* 1986;40:13-20.
 - 21 Halliwell B, Gutteridge JMC. Free Radicals and antioxidant protection: mechanisms and significance in toxicology and disease. *Human Toxicol* 1988;7:7-13.
 - 22 Travis J, Salvesen G. Human plasma proteinase inhibitors. *Ann Rev Biochem* 1985;52:655-709.
 - 23 Schein M, Saadia R, Decker GGA. The open management of the septic abdomen. *Surg Gynecol Obstet* 1986;163:587-94.
 - 24 Krenzien J, Lorenz W. Scoring-Systeme für schwere intraabdominale Infektionen. *Zentrbl Chir* 1990;115:17:1065-79.
 - 25 Krenzien J, Röding H. Prognosis of perforated peptic ulcer—stratification of risk factors and validation of scoring systems in predicting the postoperative outcome. *Theor Surg* 1990;5:26-32.
 - 26 Bohnen J, Bonlanger M, Meakins JL, McLean APH. Prognosis in generalized peritonitis—Relation to cause and risk factors. *Arch Surg* 1983;118:285-9.
 - 27 Moser KH, Bouillon B, Troidl H, Köppen L. Validation of the continuous APACHE Score (CAPS) for a better prediction of outcome in surgical ICU patients. *Theor Surg* 1989;3:192-7.