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Fifth Meeting of European Shock Society
Fourteenth Annual Meeting of the Shock Society (USA)
Third Vienna Shock Form

June 2–6, 1991

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SHOCK SOCIETY OFFICE • (706) 721-2601 • FAX (706) 721-3048
Sherwood M. Reichard • Medical College of Georgia • Augusta, Georgia 30912

PRESIDENT:
John W. Holaday, Ph.D.
9610 Medical Center Drive
2nd Floor
Rockville, MD 20850
301 217-9858 FAX 301 217-9594

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Ronald V. Maier, M.D.
Dept. of Surgery, Univ. of Washington
Harborview Medical Center, ZA-16
Seattle, WA 98104
206 223-3299 FAX 206 223-3656

SECRETARY:
James A. Cook, Ph.D.
Dept. Physiology/Med. Univ. SC
171 Ashley Ave.
Charleston, SC 29425
803 792-2978 FAX 803 792-4423

TREASURER:
Thomas E. Emerson, Ph.D.
Miles Pharmaceutical
400 Morgan Lane
West Haven, CT 06516
203 937-2000 FAX 203 937-6923

EDITOR, CIRCULATORY SHOCK:
James P. Filkins, Ph.D.
Dept. Physiology/Loyola Univ. Med. Ctr.
Maywood, IL 60153
708 216-6305 FAX 708 216-6308

COUNCILLORS:
Mark G. Clemens, Ph.D. (1995)
Dept. of Surgery (Pediatrics)
The Johns Hopkins Univ. Sch. of Med.
CMSC 7-121, 600 N. Wolfe St.
Baltimore, MD 21205
410 955-6885 FAX 410 550-5314

Edwin A. Deitch, M.D. (1995)
Dept of Surgery/Shreveport Med. Center
P.O. Box 33932
Shreveport, LA 71130
318 674-6136 FAX 318 674-6141

George C. Kramer, Ph.D. (1994)
Dept. of Anesthesiology/Div. of Res.
503 Clin. Sci. G49/Univ. Tx Med. Branch
Galveston, TX 77550
409 772-1906 FAX 409 772-8895

Robert S. Rhodes, M.D. (1994)
Dept. Surg./Univ. of MS Med. School
Jackson, MS 39216-4505
601 984-5100 FAX 601 984-5107

Jureta W. Horton, Ph.D. (1993)
Dept. Surgery, UT-Southwestern Med. Ctr.
5323 Harry Hines Blvd.
Dallas, TX 75235-9031
214 688-3762 FAX 214 688-6700

James W. Holcroft, M.D. (1993)
Dept. Surgery/Univ. of California, Davis
Sacramento, CA 95817
916 734-3779 FAX 916 734-3951

Charles L. Rice, M.D.
Dept. Surgery, UT-Southwestern Med. Ctr.
5323 Harry Hines Blvd.
Dallas, TX 75235-9031
214 688-2657 FAX 214 688-6700

SCIENTIFIC PROGRAM CHAIR:
James A. Cook, Ph.D.
Dept. Physiology/Med. Univ. SC
171 Ashley Ave., Charleston, SC 29425
803 792-2978 FAX 803 792-4423

March 20, 1993

Jeannine A. Majde, Ph.D.
Scientific Office Code: 1141SB
800 N. Quincy Street
Arlington, VA 22217-5000

RE: Grant N0014-91-J-1812

Dear Dr. Majde:

On behalf of the Scientific Program Committee, Officers and Council of the Society, I want to thank the Office of Naval Research for your support of the Symposia held at the 28th National Meeting of the Society for Leukocyte Biology, September 28-October 1, 1991 at Aspen, Colorado, the Third International Workshop on Cytokines, November 10-14, 1991 in Stresa, Italy and the Fourteenth Annual Meeting of the Shock Society held in conjunction with the Second International Conference on Shock, June 2-6, 1991 in Vienna, Austria. These meetings were informative and scientifically successful. We are grateful for the support of the Department of the Navy which helped make these meetings possible.

I am enclosing a copy of the "Journal of Leukocyte Biology" which contains the program (pages 3-15) and abstracts (pages 15-110) for the 28th National Meeting of the Society for Leukocyte Biology. The special research awards are listed on page 113 (copy of this page is enclosed). A summary of the Conference by John Cambier, Program Chair, is enclosed.

A SUMMARY OF THE JOINT 28TH NATIONAL MEETING OF THE SOCIETY FOR LEUCOCYTE BIOLOGY AND THE 21ST LEUKOCYTE CULTURE CONFERENCE

John Cambier

The intellectual focus of this meeting was aptly described by its title "Receptors and Signal Transduction in Leukocyte Biology". The program was developed to address the focus in a longitudinal fashion beginning with a keynote address and a plenary session dealing with receptor structure, proceeding to a second plenary session dealing with second messenger generating systems, and concluding with a plenary session addressing regulation of gene expression. These major sessions were complemented by mini-symposia in various more focused areas. While a plenary session was held each morning, four minisymposia were scheduled concurrently on each afternoon of the meeting. Finally, early each morning a poster session was held to provide an opportunity for students and fellows to present their data "one on one". A specific discussion of the information exchanged in these sessions is discussed below.

The meeting was opened with a keynote address by Dr. Richard Klausner who gave an excellent presentation covering mutational analysis of the T cell antigen receptor. Studies were discussed which showed that a small sequence motif found in ζ chain and CD3ϵ contains all structure information necessary for signal transduction. Dr. Klausner also described a novel approach, developed in his laboratory, for producing biologically active soluble T cell receptors. The studies described were published subsequently in two papers in Science. Dr. Klausner's presentation established a tenor which was sustained throughout the meeting. Findings presented in the first plenary session demonstrated compartmentalization of function in distinct structural motifs within Fcϵ, IFNγ, $F_c\gamma$RII and B cell antigen receptors. These excellent presentations by Drs. Kinet, Cambier, Schreiber and Mellman were well attended and very much appreciated. The minisymposia appended to this plenary session broadened the coverage of other receptors, addressing complement receptors, cytokine receptors, adhesion molecules and receptor-cytoskeletal interactions.

The second plenary session and its appended minisymposia dealt with the next step in signal transduction, the activation of cytosolic effector molecules which mediate signal propagation. This plenary session focused on tyrosine kinases and phospholipase C which play central roles in signal propagation in many receptor systems. Drs. Bolen, Schlessinger and Rhee gave superb presentations featuring new data. Unfortunately, Dr. Charles Sherr an additional scheduled speaker could not attend due to illness.

Minisymposia covered areas of cell contact mediated signaling and the role of kinases, phosphatases and ion movements in cell activation. A final minisymposium dealt with issues of signal transduction peculiar to neutrophil function - a longstanding interest of the Societies.

On the final day of the meeting, the program focused on the most distal event of signal transduction, integration of signals leading to biologic responses including activation of gene transcription. In the plenary session, chaired by Dr. Warner Greene, various model systems of transcriptional regulation were discussed. Dr. Greene described NF-kB and its role in T cell signaling. Dr. Staudt described the role of octamer binding proteins in gene activation in B lymphocytes. Dr. Michael Karin discussed positive and negative control of AP-1 activity. Finally Dr. Laurie Glimcher discussed regulation of genes encoding class II major histocompatibility antigens and presented findings of functional studies of a recently produced MHC class II knockout mice. These findings were extremely interesting, demonstrating deficits in $CD4^{+}$ T cell development and immune competence. The minisymposia scheduled after the third plenary session focused on subjects, which were more peripheral to the theme of the meeting but of traditional interest of members of the society. These sessions address killer cell-target recognition and signal transduction, macrophage anti-microbial activity, macrophage activation and regulation, and G-proteins and receptor coupling in leukocytes. These sessions provided an important opportunity for the attendees with interests primarily in biologic questions to integrate their studies into the more molecular focus of the meeting.

In closing, this was considered by most of the nearly 500 attendees to be an excellent meeting. The program schedule worked well, fostering optimal exchange of information. It was the consensus of the attendees that their attendance was well justified by the information gained.

SUMMARY OF THE THIRD INTERNATIONAL CYTOKINE WORKSHOP

Alberto Mantovani

The Third International Cytokine Workshop was held in Stresa, Italy, November 10th to 14th, 1991 with 485 participants from Europe, USA and Japan. Europeans accounted for around 60% of the participants with less than 10% Italians. 439 abstracts were submitted and these were discussed in oral sessions or posters. The Workshop consisted of 10 lectures, 8 symposia and 9 topic discussions. Emphasis was on proferred papers, in order to get the most recent exciting "news" from laboratories around the world, and these were selected by the organizers and reviewers. Personal interaction among participants was promoted by having meals in the same hotels, independently of lodging. The program was very intense with no space for recreational activities.

The Workshop was largely focused on cytokines that regulate inflammation and tissue repair starting from the first lecture of M. Sporn. Of mediators such as IL-1, TNF and IL-6, discussion included gene structure and regulation, receptors, signal transduction. Considerable interest was focused on antagonists, such as IL-1ra and soluble receptors (IL-1, TNF, IL-6). Analysis of these mediators in pathophysiology shows how they play a central role in a variety of disease processes, including allergy, inflammation and septic shock. It was stimulating and encouraging to see how cytokines are passing from experimental studies to clinical investigation and therapy. In this context, the initial clinical results with IL-1ra in septic shock were a major source of excitement. In addition to "classic cytokines" the Workshop discussed new cytokines, such as IL-12, CP10 and molecules related to IL-8.

Nomonclature is a source of confusion within and outside this area of research. This is particularly striking for the superfamily of mediators related to IL-8. The workshop provided the opportunity for a meeting of 15 scientists active in this field to discuss a review of nomenclature. They agreed on a proposal of a new name (chemokines) and classification. If accepted, the Stresa nomenclature will put an end to 2 years of debate and confusion.

This was a excellent Workshop with state-of-the-art presentations on the pathophysiology and pharmacology of cytokines. Scientists and students from 23 countries contributed to a wonderful and exciting interchange of information and techniques.

SUMMARY OF THE FOURTEENTH ANNUAL MEETING OF THE SHOCK SOCIETY

John Holaday

At the Fourteenth Annual Meeting of the Shock Society, a comprehensive program was devoted to state-of-the-art presentations defining the mediators of circulatory shock as well as promising therapeutic strategies for its treatment. There are many causes of circulatory shock, including bacterial infections, acute hemorrhage, anaphylactic responses to insect bites, and even neurogenic causes. Collectively, these different forms of shock share a common underlying problem: too little blood flow to critical organs. As we have recently learned, many biological mediators are activated during shock, and the dyshomeostatic state that characterizes this hypoperfused state is a result of the collective effects of these mediators as they are expressed over time. Of course, each of the forms of circulatory shock also differ in critical ways that require separate approaches toward understanding their etiology and treatment.

Septic shock remains as a major killer despite decades of research directed at uncovering its cause and potential cure. According to statistics reported in the United States, one patient out of every two hundred hospital admissions develops septicemia; of these, 25-40 percent die. Sepsis is also a major medical problem in managing military casualties where infected wounds can frequently result in septic shock. Despite the promise of ever more sophisticated technology, representing an exponential growth in biomedical knowledge, the clinical application of this technology has, as of yet, failed to significantly affect the morbidity and mortality of septic shock.

Septic shock from bacteria can result from the proliferation of either gram-negative or gram-positive organisms, however gram-negative sepsis predominates. An obvious way of treating septic shock is to kill the offending organisms, and the prophylactic and therapeutic use of antibiotics has represented one key milestone in the treatment of septic shock. Paradoxically, however, the use of antibiotics to kill the offending organisms may even exacerbate the shock syndrome through the release of endotoxins and exotoxins from the dying bacteria. Endotoxins from gram-negative bacteria, chemically referred to as lipopolysaccharides, activate a broad spectrum of mediators that contribute to septic shock.

In septic shock, the spectrum of signs and symptoms that have been collectively referred to as the "sepsis syndrome" are brought about by the severe disruption of normal cell, tissue and organ function. The complexity of septic shock involves a cascade of events that evolve over time, beginning with the proliferation of offending organisms and followed by the release of mediators from the bacteria and, in turn, from the infected host. As more mediators become progressively involved, the dyshomeostatic state resulting from septic shock may ultimately become irreversible.

Recent developments in our understanding of cytokines, protein-like mediators of inflammation and immune function that are released by leukocytes, were revealed at the Shock Society meetings in Vienna. Antibodies against these cytokines, including anti-interleukin-1 antibodies and anti-tumor necrosis factor antibodies, were shown to successfully block the development of septic shock. Additionally, the first symposium describing clinical efficacy of monoclonal antibodies against lipopolysaccharide endotoxin was held at these meetings.

It is perhaps unfortunate that the process of scientific research emphasizes new discoveries, often prematurely dismissing the old. Today, state-of-the-art research in circulatory shock emphasizes cytokines, monoclonal antibodies, eicosanoids and other established mediators. The Fifteenth Annual Shock Society meeting, to be held this June in Alabama, promises to provide an even more challenging program, where an integration of knowledge about these mediators will be presented. Through these meetings, the world leaders in preclinical and clinical research directed at uncovering the causes and cures for circulatory shock will present their findings. Importantly, in order to ensure the sustained enthusiasm in the research community for circulatory shock research, the Shock Society emphasizes the participation of young investigators. In fact, the young investigator award committee reviewed over forty abstracts for this year's competition. The active program of scientific exchange conducted by the Shock Society will continue to foster the sustained growth of knowledge in the area of circulatory shock.

I am enclosing a copy of "Cytokine" which contains the program (pages 440-448) and abstracts (pages 450-521) for the Third International Workshop on Cytokines. A summary of the Workshop by Alberto Mantovani, Program Chair, as well as the published Proceedings of the Workshop, are enclosed.

I am also enclosing a copy of "Circulatory Shock" which contains the program (pages 1-6) and abstracts (pages 7-170) for the Second International Conference on Shock. A Summary of the Conference by John Holaday, U.S. Program Chair, is enclosed.

This report has been delayed in order to include the program for the Vienna Conference of Shock which was reprinted, and the publication of the Proceedings of the Cytokines Workshop.

I look forward to a continued association of the Society with the Office of Naval Research.

Sincerely,

Sherwood M. Reichard
Executive Director

SMR/ljh

enclosures

THIRD INTERNATIONAL WORKSHOP ON CYTOKINES

SUPPORT

·ganizing Committee of the Third International Workshop on Cytokines would
:o thank the following institutions and companies for their major grants.
ıt their valuable help this conference could not have been organized.

Banca Popolare di Novara

Commision of European Communities

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Office of Naval Research

Society for Leukocyte Biology

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SOCIETY FOR LEUKOCYTE BIOLOGY AWARDS

PRESIDENTIAL AWARDS

President Dolph O. Adams is pleased to announce that the first place winner for the Presidential Award of the Society this year will receive a plaque and a Cash Award of $600. This will be given for the best student paper presented at the Twenty-Eighth National Meeting of the Society for Leukocyte Biology. The second place award will be $300 and a plaque. Competition for this award will include all "candidates in training" (predoctoral and postdoctoral) with a maximum of two years of postdoctoral work. Recipients must be a member of the Society for Leukocyte Biology.

The first place award is funded by the Office of Naval Research and the second prize by the Annie R. Beasley Memorial Fund. Winners will be announced at the Banquet, Monday, September 30, 1991.

1991 AWARD FINALISTS:

Tatiana M Oberyszyn, UMDNJ-Robert Wood Johnson Medical School
Jan M Schultz, University of Wisconsin Medical School
Steven Weinstein, University of California, San Francisco
Andrew Yurochko, University of North Carolina

YOUNG INVESTIGATOR AWARD

This competition is open to all investigators under 36 years of age. Must be a member of the Society for Leukocyte Biology or sponsored by a member. The first place award is $600 and a plaque.

1991 AWARD FINALISTS:

Richard R Kew, SUNY at Stony Brook
Elizabeth J Kovacs, Loyola University
Carl L Manthey, U.S.U.H.S.
Anne M Pilaro, NCI-FCRDC

RES RESEARCH AWARD

The Society for Leukocyte Biology will present the Marie T. Bonazinga Annual Research Award at the Twenty-Eighth National Meeting. This prestigious award is sponsored by the Accurate Chemical and Scientific Corporation and is to be presented to a member of the Society who has demonstrated excellence in research. Presentation of the $3,000 award will be made at the banquet on Monday, September 30, 1991.

The 1991 AWARD WINNER is:

Zanvil A. Cohn
Professor and Senior Physician
Lab of Cellular Physiology and Immunology
The Rockefeller University
1230 York Avenue
New York, NY 10021

MEETING SUPPORT

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Second International Conference on Shock
Fifth Meeting of European Shock Society
Fourteenth Annual Meeting of the Shock Society (USA)
Third Vienna Shock Form

June 2–6, 1991
Congress-Site: Vienna Hilton am Stadtpark

Organizers:

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The Shock Society (USA)
in association with the Japanese Shock Society
and the International Endotoxin Society

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PROGRAM

Sunday June 2, 1991

2:00 pm
Council Meeting (Shock Society (US))
4:00 pm
Council Meeting (European Shock Society)
4:00–7:00 pm
Registration
7:00–10:00 pm
Reception

Monday June 3, 1991

9:00 am
Opening Ceremony
Presidential Address: G. Schlag (Europe)
I.H. Chaudry (USA)
H. Ogata (Japan)
E. Rietschel (Int. Endotox. Soc.)

9:45 am
Perutz M., Nobel Laureate: Molecular Mechanism of Oxygen Transport

10:30 am–12:30 p.m.
Plenary Session: Polytrauma (No 1–4)
Chair: F. Lewis — R.J.A. Goris

1) *Trauma in the 90's: How far have we come; Where are we going?*
F. Lewis
San Francisco, CA, USA
2) *Clinical Manifestation of General Cell Damage after Severe Trauma*
J.A. Sturm
Hannover, Germany
3) *Integral Management of the Multiple Traumatized Patient*
R.J.A. Goris
Nijmegen, The Netherlands
4) *Cardiovascular Response to Haemorrhage and Injury*
R.A. Little
Manchester, United Kingdom

12:30–2:00 pm
Poster Session (with Buffet Lunch)
(No 13–21, 29–48, 55–56, 63–74, 81–83, 91–102, 110–113)

Symposia
S9. Neuro-Endocrinological Systems (No 144–149)
S10. Models of Organ Failure and Sepsis (No 161–171)
S11. Biological Monitoring (No 200–212)
S12. Endotoxin and Cytokines (No 245–256)

3:45–4:15 pm
Coffee
4:15–6:00 pm
Symposia
S10. Cont. Models of Organ Failure and Sepsis
S11. Cont. Biological Monitoring
S12. Cont. Endotoxin and Cytokines
S13. Update of Metabolism (No 267–273)

Wednesday, June 5, 1991

6:30 am
Presidential Run—Hotel Departure
8:30–9:15 am
R. Huber, Nobel Laureate: Proteolytic Enzymes and their natural inhibitors, models for biophysics and biochemistry and targets for drug design
9:30 am–12:30 pm
Plenary Session:
Therapeutic Interventions—Sepsis and Organ Failure (No 286–290)
Chair: O. Trentz — R.C. Bone
9:30–10:30 am
Therapeutic Interventions—Sepsis and Organ Failure
O. Trentz
Zürich, Switzerland
Therapeutic Interventions: Sepsis and Organ Failure
R.C. Bone
Chicago, Illinois, USA
Prevention of Multiple Organ Failure by SDD
Ch. Stoutenbeek
Amsterdam, The Netherlands
10:30–11:00 am

Coffee

11:00–12:30 pm

Imbalance Between Oxygen Demand and Oxygen Supply
J.-L. Vincent
Brussels, Belgium

The Use of Antiendotoxin Monoclonal Antibody to Treat Gram-Negative Bacteremic Shock
E.J. Ziegler
San Diego, CA, USA

12:30–2:00 pm

Poster Session (with Buffet Lunch)
(No 213–244, 274–285, 297–298, 313–319, 326–328, 339–342, 350)

2:00–3:45 pm

Symposia

S14. Surgical Approach
(No 291–296)

S15. Vasoactive Drugs—Circulatory Support
(No 299–312)

S16. Extracorporeal Elimination
(No 320–325)

S17. Anti Endotoxin Measures and Immunoglobulins
(No 329–338)

3:45–4:15 pm

Coffee

4:15–6:00 pm

Symposia

S15. Cont. Vasoactive Drugs—Circulatory Support

S17. Cont. Anti Endotoxin Measures and Immunoglobulins

S18. Antibiotics and Gut Decontamination
(No 343–349)

S19. Antiproteases and Miscellaneous Drugs
(No 351–356)

Thursday, June 6, 1991

8:30–9:15 am

Ch. Gelin Memorial Lecture—A.E. Taylor
The Use of Animals in Research: Benefits to Human and Animal Welfare

9:15–11:15 am

Plenary Session: Young Investigators Award
F.R. Lewis — R.A. Little
ESS (No 369–373)
+ Shock Society (US) Finalists

11:15–11:45 am

Coffee — (Jury Meeting)

11:45 am

Awards Ceremony

12:30–2:00 pm

Poster Session (with Buffet Lunch)
(No 357–368, 380–386, 395–400, 416–440, 448–459, 467–469)

2:00–3:45 pm

Symposia

S20. Platelet Activating Factor Antagonists
(No 374–379)

S21. Xanthines—Multifunctional Therapeutic Agents
(No 387–394)

S22. Free Radical Scavengers
(No 401–415)

3:45–4:15 pm

Coffee

4:15–6:00

Symposia

S22. Cont. Free Radical Scavengers

S23. Eicosanoids—Generation and Inhibition
(441–447)

S24. Antibody Therapy against TNF, and Adherence
(No 460–466)

Second International Conference on Shock
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Abstracts

Plenary Session: Polytrauma

Notes

INTEGRAL MANAGEMENT OF THE MULTIPLE TRAUMATIZED PATIENT. R.J.A. Goris. University Hospital Nijmegen, P.O.Box 9101, 6500 HB Nijmegen, The Netherlands

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The chances of survival and rehabilitation of a severely injured patient depend on (i) fixed factors, such as the severity of injuries and the general condition of the victim prior to the accident and (ii) variable factors, including the immediate availability and quality of pre-, in- and post-hospital trauma care. In the acute phase all efforts are aimed at identifying and treating disturbances of vital functions (eg. clearing the airway, restoring and securing optimal ventilation and circulation). Hypoventilation is common and often unrecognised, especially in the brain-injured. Positive pressure ventilation should therefore be instituted early. As shock is almost uniformly due to hypovolaemia, vigorous fluid administration is started immediately. From the onset, the patient should be monitored intensively as in the ICA. After identification and operative control of all accessible bleeding sites and blood transfusion according to requirements, the respiratory and cardiovascular functions should stabilise. By then, more extensive diagnostic procedures should be undertaken, followed by a series of less urgent operations. All major fractures should be stabilised the day of injury. Following this diagnostic period which should not last longer than one hour, a second set of operative procedures is performed, including treatment of open fractures, joint injuries, peripheral vascular injuries and compartment syndromes to prevent ARDS and late death from sepsis. Allowing respiratory failure to develop before providing optimal respiratory support leads to a higher incidence of ARDS which tends to be more severe.

CARDIOVASCULAR RESPONSE TO HAEMORRHAGE AND INJURY. R.A. Little and E. Kirkman, North Western Injury Research Centre, Medical School, Manchester M13 9PT, UK.

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The failure to recognise the presence and/or magnitude of post-traumatic fluid loss is still a cause for concern (1). One hypothesis is that the clinical signs of blood loss (e.g. a change in heart rate) are modified by injury. The heart rate response to injury is biphasic: an initial baroreflex mediated tachycardia followed, as the magnitude of blood loss increases, by a bradycardia mediated by cardiac c-fibre afferents (2). This pattern of response to haemorrhage is modified in the presence of tissue ischaemia/injury such that the bradycardia is markedly attenuated (3). Further investigation has shown that the baroreflex is itself, inhibited both in experimental animals and man by injury and that this effect is mediated by stimulation of somatic afferent nociceptive c-fibres. Also the reflex bradycardia elicited by stimulation of the cardiac c-fibres is inhibited by such nociceptive stimulation (4). This modification by peripheral injury of the homoeostatic heart rate response to haemorrhage reduces the ability to withstand blood loss such that a smaller loss is required, in the presence of injury, to induce a given degree of depression of cardiac flow and oxygen transport variables. These findings confirm and help explain the observation that stimulation of nerve fibres which form the afferent pathway from injured tissues reduces survival time following haemorrhage (5).

1. Anderson, I.D. et al. (1988). BMJ 296, 1305-1308
2. Secher, N.H. et al. (1984). Circ. Shock 14, 267-274
3. Little, R.A. et al. (1989). Exp. Physiol. 74, 825-833
4. Kirkman, E. et al. (in press). Arch. Em. Med.
5. Overman, R.R. & Wang, S.C. (1947). Am. J. Physiol. 148, 289-295.

Notes

S1: Pathophysiology and Therapy of Burn Injury

5 RESUSCITATION OF THE THERMALLY INJURED PATIENT WITH AND WITHOUT INHALATION INJURY. B.A. Pruitt, Jr., W.G. Cioffi, W.F. McManus and A.D. Mason, U.S. Army Institute of Surgical Research,Fort Sam Houston, TX 78234-5012, USA.

The response of the hemodynamic system to burn injury is proportional to the extent of the burn and results from changes in cardiac and vascular function as well as loss of extravascular volume. Prompt administration of adequate resuscitation fluid corrects the circulating blood volume deficit and prevents the development of acute organ failure. Numerous formulae, recommending markedly different volumes and types of resuscitation fluid, have all been found to be effective in the resuscitation of uncomplicated young adult burn patients.

There are subsets of burn patients, e.g., those with inhalation injury, who require individualized management of their resuscitation regimen. The anticipated increased susceptibility to pulmonary edema of patients with inhalation injury would speak for reducing the volume of resuscitation fluid infused. To the contrary, however, both laboratory and clinical studies have demonstrated that burn patients with inhalation injury typically have increased resuscitation fluid needs that must be met to reduce the impact of the combined injury. Hemodynamic monitoring of those and other complicated burn patients enables one to manipulate the resuscitation regimen and utilize pharmacologic agents to maintain vital organ function at the least immediate or delayed physiologic cost.

6 EDEMA FORMATION IN BURNS. Aubrey E. Taylor, Lynn Dyess, J. Collins, and R. Fletcher, University of South Alabama College of Medicine, Mobile, AL 36688 USA

Recent studies from our laboratory have indicated that burns not only cause endothelial damage, but in addition the capillary pressure is increased to levels approaching arterial pressures. Since the loss of fluid from the plasma into the interstitium following burn injury is directly related to the product of the increased capillary pressure and a capillary water permeability factor, it is not surprising that it is difficult to maintain a plasma volume that is sufficient to properly oxygenate the body tissues. However, when evaluating the lymph flow dynamics after paw-burn, we found very unexpected findings which can best be appreciated by the following data:

	PAW WEIGHT			LF
	CONTROL		AFTER BURN	
OBSERVED (6 hrs)	180 gm	(90)	270 gm	220 gm
PREDICTED (6 hrs)	180 gm	(220)	400 gm	90 gm

The limb only gained 90 gms over 6 hours, but would have gained 220 gm if lymph flow did not increase to high levels. The possible mechanisms responsible for these huge lymph flows will be discussed in terms of edema dependent lymphatic factors such as 1) decreased tisue resistance; 2) altered tissue compliances; 3) protein stimulation of the lymphatic pumping activity; 4) compounds released by the endothelium; 5) the inflammatory response and 6) neutrophils somehow affect the lymphatic pump; and 7) the baroreceptor reflex causes sympathetic stimulation of the lymphatics which can alter their pumping capabilities.

7 PULMONARY EDEMA FOLLOWING INHALATION INJURY. Daniel L. Traber. Univ. of Texas Med. Branch and Shriners Burns Institute, Galveston, TX 77550, USA.

Inhalation injury is a dominant cause of death in victims of fire accidents. A major contributor to the seriousness of this problem is related to pulmonary edema. We have studied this in both animals and man. The *ovine* chronic lung lymph preparation is a good model for these studies. Lung lymph flow is an index of pulmonary transvascular fluid flux. This is increased some eight fold following inhalation injury. These changes develop over a six hour time period and reach a peak at 24 hours after inhalation injury. There is a concomitant increase in extravascular lung water as measured by both thermal dilution and gravimetric techniques. The formation of the lymph is associated with both an elevation in microvascular pressure and permeability. The mediators responsible for the damage appear to be associated with neutrophils. These cells are found in increased numbers in the lung tissue, lavage fluid and lymph following inhalation injury. These cells release O_2 radicals and proteolytic enzymes after margination. Conjugated dienes are increased in the lung lymph and plasma following smoke inhalation and the injury is not as great if the animals are treated with O_2 radical scavengers. The antiproteases are likewise reduced after inhalation injury and the lung lymph flow is less elevated after treatment with an antiprotease. There is evidence that chemical injury to the trachea may be responsible for the release of mediators that attract the neutrophils to the lung. (Supported by NIH Grants GM33324 and HL37411 and a grant from the Shriners of North America).

Notes

ALTERATIONS OF NONSPECIFIC AND SPECIFIC HOST DEFENCE IN BURNED PATIENTS. W.König, J.Brom, B.Schlüter and M.Köller.
AG Infektabwehrmechanismen, Ruhr-Universität Bochum, D-4630 Bochum 1

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We investigated the pathophysiological alterations in severely burned patients and analyzed 1.)microbial colonization and invasion in bioptic tissue 2.)the role of microbial pathogenicity factors 3.)the response of patients' granulocytes towards physiological stimuli and 4.)cytokine expression and release. Within bioptic tissue S.aureus and P.aeruginosa with different pathogenicity pattern are predominant. During microbial invasion the granulocytes loose their ability to generate and metabolize lipid mediators (e.g.leukotrienes, PAF) due to severe changes in signal transduction cascade (e.g. G-proteins, 5-lipo-oxygenase). These alterations can be partly mimicked in an experimental stress model ("heat shock"). Cells of burned patients showed reduced HSP expression as compared to normal cells. Nonsurvivors as compared to survivors revealed enhanced IL6 levels, also increased IL6 mRNA expression, enhanced CD25 and reduced CD23 expression. Our results clearly show early occurring differences in host defence for surviving and nonsurviving patients which may determine microbial invasion and fatal outcome.

ADVANCES IN THE THERAPY OF MAJOR BURN INJURY. DN Herndon, DL Traber, RL Rutan* and TC Rutan*.
Shriners Burns Institute and University of Texas Medical Branch, 610 Texas Ave., Galveston, TX 77550.

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Advances in the treatment of major burn injury have led to dramatic improvements in survival. About 1/2 the patients ≤35 years of age can now survive burns of 90% total body surface area (TBSA). However, a concomitant smoke inhalation injury greatly decreases survival rates. Resuscitative fluid needs in combined smoke inhalation and burn injury are almost 2 ml/kg/%TBSA/day greater than with burn injury alone. Research in a sheep model has demonstrated a marked neutorphil diapedesis and margination within the pulmonary vasculature with a secondary release of proteolytic enzymes which increase microvascular permeability leading to lung edema. Tracheobronchial destruction with resultant cast formation leads to atelectasis. New therapeutic approaches using nebulized heparin, free oxygen radical scavengers (N-acetyl-cysteine), systemic thromboxane synthetase inhibitors and proteolytic enzyme inhibitors (gabexate mesilate) have been efficacious in aminal models and are now in clinical trials. Modulation of the endocrine mediators of the hypermetabolic response to burn trauma have also demonstrated clinical efficacy. Propranolol decreases heart rate and anxiety without adverse metabolic effects. Use of recombinant human growth hormone has increased nitrogen synthesis, decreased nitrogen breakdown, increased wound healing and decreased length of hospital stay. Autologous, tissue culture grown epithelial cells have allowed the salvage of even the largest of burns. Current research on multiple growth factors (EGF, PDGF, FGF) should improve quality of skin and scar and thereby quality of life.

IMPROVEMENT IN ILEAL AND HEPATIC MORPHOLOGY AFTER BURN INJURY WITH EPIDERMAL GROWTH FACTOR. J. Hansbrough*, R. Zapata-Sirvent*, P. Wolf*.
Univ. California, San Diego 92103

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An important function of the GI tract is to exclude microorganisms and endotoxin from the systemic circulation. This property may be altered by certain conditions such as the presence or absence of intraluminal nutrients, by changes in intestinal flora, and by injury. Alterations of GI mucosa may lead to bacterial translocation (BT), which may increase infectious complications after injury. The pathogenesis of alterations in the GI tract following injury remains to be elucidated. We employed a burn mouse model in which a 25% TBSA full-thickness burn was induced by exposing the depilitated dorsum to steam for 6 seconds. Mucosal cells and hepatocytes are known to have surface receptors for epidermal growth factor (EGF). Burned animals were fasted and were administered 4 ug EGF i.p. at 1 and 12 hrs postburn, to determine if this trophic hormone would protect the GI tract and the liver after injury. At 24 hrs, liver and small intestine was removed and sections of distal ileum and liver were processed in paraffin, sectioned and stained with H&E. Mucosal and villous height were analyzed by light microscopy. Severe alterations of ileum morphology were observed 24 hrs postburn in fasted animals, with shortening, degeneration and atrophy of the villae. Swelling of hepatocytes and pyknotic nuclei were also seen, with edema and obliteration of sinusoids of the liver. Administration of EGF largely prevented the morphologic alterations of the ileal mucosa and liver, and increased ileum weight and villous height. **CONCLUSIONS:** After burn injury, severe alterations of the GI mucosa and liver are seen acutely. These morphologic alterations can be almost completely prevented by administration of EGF postburn. Maintenance of the barrier function of the GI tract mucosa may prevent BT and decrease infectious complications after burn injury; EGF may also prevent acute damage to hepatocytes after burn injury.

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11 LEUKOTOXIN, 9,10-EPOXY-12-OCTADECENOATE, AS A BURN TOXIN

Takayuki Ozawa, Mika Hayakawa, Kazuhiro Kosaka, Satoru Sugiyama, Kazuhisa Yokoo, Hisashi Aoyama, and Yohei Izawa
Department of Biomedical Chemistry, Faculty of Medicine, University of Nagoya, Tsuruma, Showa-ku, Nagoya 466, Japan.*

It is postulated that toxic substances (burn toxin) synthesized in burned skin are transferred into general circulation and cause multiple organ failure. We have found that a linoleate epoxide, 9,10-epoxy-12-octadecenoate, biosynthesized by leukocytes is an antagonist to EDCF, endothelin, leading to vasodilation at its 1μM. At the case of serious inflamation, plasma concentration of the epoxide frequently exceeds 10μM where the epoxide is highly cytotoxic, thus nominated as leukotoxin. Leukotoxin exists in patients' plasma as well as in burned skin. To evaluate the possibility of leukotoxin as a burn toxin, we studied plasma leukotoxin level of four patients with extensive burns (over 50% of body surface area) and examined coagulation studies in these patients. We detected considerable amounts of leukotoxin (11.4 μM - 37.0 μM) in all patients. Leukotoxin was below 2μM in the control subjects. Pulmonary edema, cardiac failure, and coagulation abnormalities were found in these patients. Exogeneously administered leukotoxin induced similar pathological conditions in experimental animals to those observed in patients with extensive burns. Hence, it is concluded that leukotoxin is a responsible substance to shock and multiple organ failure, as a burn toxin.

13 CHANGES IN CORE TEMPERATURE IN THE EARLY POST-BURN PERIOD IN ADULTS.

L. Martineau*, R.A. Little, I.T. Campbell, P.J. Davenport*, and N.J. Rothwell*. Regional Burns Unit, University Hospital of South Manchester, Manchester, U.K. M20 8LR.

Several animal studies have shown that body temperature is reduced immediately following burning injury. In contrast, it has been observed that children and infants become markedly pyrexic within only a few hours after being burned (1). There are no data to determine the direction of these changes in burned adults. This study reports the pattern of core temperature (T_c) of 17 adults (17-63 years of age) during the first 36 h after burn injury covering 14-45 percent of the body surface area (BSA). All patients but two required intravenous resuscitation. On admission, T_c measured 1-5 h after the injury ranged from 34.1 to 37.8°C, only three patients having T_c<36°C. T_c remained fairly stable for the first 36 h in patients with burns covering <24% of BSA, averaging 37.4±0.1°C. In contrast, T_c rose gradually to 38.3±0.3°C during the first 12 h in 7 patients with burns covering 41±2% of BSA, T_c remaining constant throughout the next 22 h. There was no relationship between the peak core temperature reached during the first 36 h post-burn (T_{peak}) and either the sex of the patient or the ambient temperature. The severity of the injury was a major variable to determine T_{peak}, as suggested by a strong correlation between T_{peak} and the total percentage of BSA burned ($p<0.001$; $r = 0.91$). There was also a negative correlation between T_{peak} and age ($p<0.01$; $r = 0.73$). These data show that there are marked age-related changes in the acute thermal response to burning injury in man.

1. Childs, C. (1988). Burns 14, 1-6.

14 CHANGES IN OXYGEN CONSUMPTION AND HEAT LOSS PROVIDE FURTHER EVIDENCE FOR AN INAPPROPRIATE THERMOREGULATORY RESPONSE DURING THE ACUTE PHASE OF BURN INJURY IN CHILDREN.

C. Childs[1,2], H.B. Stoner[2] and R.A. Little[2]
Regional Paediatric Burns Unit, Booth Hall Hospital[1] and University of Manchester, NWIRC[2], Oxford Road, Manchester M13 9PL, UK.

The early rise in rectal temperature (T_r) in burned children during the first 24 h after injury correlates well (r=0.77, n=24) with elevated levels of the circulating endogenous pyrogen (EP), Interleukin-6 (IL-6) (1). The central effect of EP is to raise the hypothalamic set-point and thus the threshold temperatures for the onset of heat production (HP) and heat loss (HL). We have shown that in children acutely after burning, there is an inhibition of sweating, and reduced heat loss in acral regions at Ta 30°C; an environmental temperature at which heat loss is increased in controls. Little is known about HP at this time. Serial measurements of oxygen consumption ($\dot{V}O_2$, n=91) were made, by indirect calorimetry in 10 patients aged 12m-10y during the first 24 h after 10-60% burns and in 40 healthy children (4wm-13y) under similar environmental conditions (bandaged and at Ta 30°C). $\dot{V}O_2$ in sedated febrile patients was significantly higher than in afebrile sleeping controls but not different from resting controls. The pattern of change in $\dot{V}O_2$, heat loss and Tr acutely after burn injury in children indicates that the early pyrexia is a consequence of an upward resetting of the thermoregulatory set-point.

1. Childs, C. et al. (1989). Cytokine 1, 136.

Notes

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THE IMPORTANCE OF CONCOMITANT DESEASES, RISK FACTORS AND INHALATION INJURY FOR THE PRECISION OF SCORING SYSTEMS IN BURNS.

Germann, G., Kuipers, T., Barthold, U., Perbix, W.

Klinik für Plastische Chirurgie, Wiederherstellungs- u. Handchirurgie, Schwerstverbranntenzentrum, Köln-Merheim

Scoring systems in burn patients are still lacking precision in specifity. This is caused by underestimating the role of certain risk factors for the outcome of burned patients.
Methods: 450 patients were analyzed for validation of the mod. Baux and Tobiasen Score. Concomitant diseases, risk factors as alcohol or nicotin history and inhalation injuries were computed and unifactorial as well as multivariance tests were performed.
Results: Alcohol and nicotin are significant risk factors in patients burned in mod. Baux group 70-105 or Tobiasen 7-9. These are borderline groups, where certain stress factors may lead to a fatal outcome for the patient. Most important factor is inhalation injury in all severity groups. Heart and neurological diseases were significant only in groups 70-95 or TOB 7-9. Suicidal patients have an extreme risk of death (49%).
Conclusion: Additionally to the data found in the literature, alcohol and nicotin are identified as major risk factors in a large, well documented burn population. Widely used scores under estimate the role of existing risk factors. Specifity, esp. in borderline cases, could be improved by incorporating these risk factors.

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SEQUENCE, INCIDENCE AND INTERACTION OF SEPTIC ORGAN FAILURE IN SEVERELY BURNED PATIENTS.

Germann , G., Perbix, W., Janshoff, G., Kuipers, T., Schwerstverbranntenzentrum, Klinik für Plastische Chirurgie, Wiederherstellungs- u. Handchirurgie , Köln-Merheim

Sepsis is the cause of death in 75% of all fatal cases in burn ICUs. Multiple organ failure as ARDS or kidney failure are predominantly caused by sepsis.
In 120 patients requiring long term ventilation therapy we analyzed incidence sequence and interaction of different organ failures.
Methods: Functional parameters were evaluated for lung, kidney, liver, heart/ circulation, coagulation and bone marrow. Lactat and bateriological results were computed additionally.
Results: Mortality was 52,5% in the study group. Sixty-seven patients demostrated 84 periods of organ dysfunction. 49 patients had an initial inhalation trauma, only 15 patients survived a 3 OF. Organ dysfunction mainly started with ARDS (75% of all MOF) followed by circulatory insufficiency (20%). Only 5% of the patients with MOF had initial kidney failure.
Twenty five patients showed 1 and 2 OF with a mortality of 25 resp. 35%. All patients had septic signs prior or at the time of onset of OF!!.
Time of onset is day 5-6 postburn for the lung and day 7-9 for kidney failure. Circulatory dysfunction combined with general septic signs is often found on day 4-6. Fourty % blood cultures were positive (75% gram negative).
Conclusion: Results demonstrate a possible interaction between septic complications depending on the timely interval of the onset. Ongoing analyses try to identify single septic signs as prognostic findings for early clinical diagnoses of impending OF.

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NONINVASIVE ESTIMATION OF PULMONARY HYPERTENSION IN BURNED PATIENTS WITH ACUTE RESPIRATORY FAILURE. DISCRIMINANT CRITICAL VALUES.

K. Szabó G. Jokkel, R. Pick. Burn Center of Central Mil. Hosp., Budapest, and Exp.Res.Inst. and 2nd Dpt. of Physiolog. of Semmelweis Med.Univ.School. Budapest, Hungary.

Determination of cardiac index and pulmonary arterial blood pressure: Swan-Ganz catheter, P32 Statham or DPT3003 PVB transducer, cardiac output computers (Gould MB1000, or Marquette 7010) and extended systolic time interval measurements complited by PC program package (Szabó et al. 1987) were performed simultaneously in 7 burned patients with respiratory failure at 38 occasions. The values of cardiac indices with the two methods were practically the same: 3.4±1.21/3.1±1.10 l/min./m2: regression equation: CIs=0,887*CIt=0,137, r=0,976, n=38. Close correlations have been found between PAP values and PaO2/FiO2 versus some noninvasively measured hemodynamic data. Using these interrelations:
1. regression equations for PAPs, PAPm, PAPd, PCWP, PVRI were eleborated (r values: 0,855: 0,869: 0,681: 0,644: 0,8917 respectively).
2. discriminant analysis with noninvasive parameters correctly classified the cases at critical PAPd-PCWP (>4mmHg) in 84%.
3. critically high pulmonary arterial systolic pressure (>40mmHg) was also classified correctly with noninvasive parameters in 92% of the cases.
These results suggested that a PC aided noninvasive cardiac monitor would be able to estimate continuously the pulmonary arterial pressure values, avoiding the potential infectious risks of the long-term indwelling pulmonary arterial catheterization.

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EFFECT OF THERMAL INJURY, TNF (TUMOR NECROSIS FACTOR), PAF (PLATELET ACTIVATING FACTOR), OR SUPERIOR MESENTERIC ARTERIAL OCCLUSION (SMAO) ON NEUTROPHIL MIGRATION TO THE LUNG

M.Trop, E.J.Schiffrin, E.A.Carter
Department of Pediatrics, University of Graz and Massachusetts General Hospital, Shriners Burns Institute, Boston

Lung injury associated with thermal trauma results in many cases in the development of adult respiratory distress syndrome (ARDS). The mechanisms are not clear but neutrophils and cytokines are thought to be involved. The purpose of this study was to determine if we could document increased neutrophil migration in a animal model, female rats, 175-200 grams, of thermal injury, and then determine if TNF, PAF or intestinal ischemia produced by SMAO could simulate the thermally induced effect.

A 20 % TBSA (total body surface area) burn injury resulted in a 2-3 fold increase in the indium-111 labelled neutrophils found in the lung of the burned animals compared to the controls. There was also a significant reduction in the spleen uptake but no effect on the liver and kidney uptake. The injection of TNF and PAF increased the uptake of the labelled neutrophils to the lung. PAF also reduced the uptake of labelled material by the spleen. The production of intestinal necrosis by SMAO resulted in a marked reduction of radioactivity in the spleen kidney and liver. However, there was no increase in the uptake by the lung.

These studies demonstrate that following the application of thermal injury to the rat, there is an enhanced uptake of indium-111 human neutrophils to the lung. This phenomenon could be reproduced by the injection of normal rats with the cytokines TNF or PAF, but could not be duplicated by the production of frank intestinal necrosis following SMAO. These data suggest that part of the mechanism whereby thermal injury results in increased migration of neutrophils to the lung may be related to the cytokines TNF and PAF, released and/or generated as a result of the thermal injury to the skin.

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INVOLVEMENT OF LEUKOTRIENES IN PULMONARY DAMAGE AFTER INHALATION INJURY.

S. Abdi*, K. Sugi*, L.D. Traber, J. Flynn, D.N. Herndon, D.L. Traber. UTMB & Shriners Burns Inst. Galveston, TX 77550

Pulmonary injury is the main determinant of survival in patients with smoke inhalation. The lipoxygenase metabolites of arachidonic acid are known to mediate the increased pulmonary fluid flux. We investigated the significance of endogenous leukotrienes (LT) following inhalation injury. **METHODS**: Sheep (n=7) were chronically instrumented with Swan-Ganz, central arterial and venous catheters. After a recovery of five days, baseline data were collected and the animals were insufflated with 4x12 breaths of cotton smoke. Data were collected for 24 hrs post injury. Lung lymph LTB4 and LTC4/D4 levels were measured by radio-immunoassay after HPLC separation. **RESULTS**: There was a significant increase in lung lymph flow (QL) and lung lymph clearance of LTB4 (cLTB4) and LTC4/D4 (cLTC4/D4) 24 hrs post injury.

Time (hr)	cLTB4 (ng/hr)	cLTC4/D4 (ng/hr)	QL (ml/hr)
0	1.8 ± 0.5	26.6 ± 9.1	6 ± 1
4	3.7 ± 1.8	44.6 ± 13.4	17 ± 4
8	6.3 ± 1.9*	78.1 ± 15.7	27 ± 3*
24	7.8 ± 2.8*	129 ± 34.3*	42 ± 5*

All values are expressed as MEAN ± S.E.M * = P < 0.05 (Dunnett's Test)

CONCLUSION: Our data suggest that the high lung lymph clearance of LTC4/D4 may play an important role in inducing pulmonary microvascular permeability changes following cotton smoke inhalation injury. (Supported by GM 33324; HL 37411)

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ONE LUNG INHALATION INJURY CAUSE EDEMA IN THE CONTRALATERAL AIR INSUFFLATED LUNG.

HM Loick, MD, CH Hurst, BS, LD Traber, RN, DN Herndon, MD, DL Traber, PhD, UNIV. TEX. MED. BR. AND SHRINERS BURNS INST., GALVESTON, TX (supported by GM33324).

The pulmonary pathology after smoke inhalation is significantly mediated by cytokines released from granulocytes (PMN) and macrophages. We hypothesized that the chemicals in smoke cause the cells of the airway to release chemotactic agents which prime attract and cause the margination of PMNs the lung parenchyma where they release cytokines and induce injury. We tested this hypothesis by insufflating one lung and the upper airway with smoke and determined whether the air insufflated contralateral lung showed PMN related damage. **METHOD.** In 6 sheep the right lung and lower trachea was insufflated with smoke. The contralateral lung was insufflated with air. Two sheep had both lungs insufflated with air. All animals were studied for 24 hours. Lungs were harvested at autopsy and examined for wet/dry lung ratio and tissue conjugated dienes (CD, a product of lipid peroxidation). Additionally the lungs of five normal animals were taken for determination of baseline conjugated dienes and wet/dry ratio.

RESULT

	Tissue Conjugated Dienes Absorbance (233 nm)			Wet/Dry Ratio		
	baseline	sham smoke	smoke	baseline	sham smoke	smoke
Right Lung	1.99±0.12	1.96±0.03	3.51±0.12*†	4.30±0.18	4.41±0.22	5.28±0.14*
Left Lung	1.84±0.15	1.75±0.01	2.54±0.17*	4.29±0.13	4.24±0.26	4.89±0.15*

Data are presented as mean±SE, *p<0.05 vs baseline, †p<0.05 between right and left lung (t-test)

Tissue CD were significantly increased both in the smoked right and in the nonsmoked lungs compared to

Notes

baseline. There is also a significant increase in wet/dry ratio in both lungs. Sham smoke alone did not cause an increase in tissue CD or edema formation, compared to baseline. **Conclusion.** The parenchymal damage to the lung seen with smoke injury appears to be mediated by the release of materials from the upper airway. These mediators appear to induce much or their damage by the release of O_2 radicals.

LOSS OF PLASMA ANTIOXIDANTS AFTER BURN INJURY. H. Gasser, E. Paul, H. Redl, G. Schlag, D. Traber* and D. Herndon*. Ludwig Boltzmann Inst. Exp. Clin. Traumatol., Vienna, Austria; * Shriners Burns Inst., Galveston, USA. **21**

There is experimental evidence that burn injury leads to cytotoxic oxygen radical formation and consequently to membrane lipid peroxidation.
Methods: 14 burn patients (mean total extension of 60 ± 17 % with 32 ± 23 % of third-degree, 8 of 14 with inhalation injury and a mortality of 60 %) were serially analyzed for lipid peroxides and antioxidants in plasma. Alpha-tocopherol was determined by HPLC. DTNB reactive total sulfhydryl (SH) groups were measured photometrically. In purified plasma lipid extracts diene conjugation was measured spectrophotometrically at 240 nm. Healthy volunteers (n = 12) were used as controls.
Results: Mean plasma alpha-tocopherol (Vit. E) levels were significantly ($p < 0.05$) lower and conjugated dienes significantly ($p < 0.05$) elevated over the whole observation period compared to controls. Total SH groups were very similar to controls on the first day and then decreased significantly ($p < 0.05$).

	Co	Burn patients (n = 14)						
	(n = 12)	1	2	3	6	8	12	14 days
Vit.E	118.30	25.60	26.00	36.90	31.90	39.90	57.10	48.30 mean
µg/g Prot	20.70	26.90	19.40	21.70	16.30	27.40	27.20	29.90 ± S.D.
c.Dienes	0.27	0.93	0.86	1.00	0.77	0.66	0.62	0.59 mean
abs/mg Lip	0.04	0.33	0.50	0.50	0.45	0.27	0.27	0.31 ± S.D.

Conclusion: Occurrence of conjugated dienes indicates lipid peroxidation. Low antioxidant levels in these patients reflect a seriously diminished defense against toxic oxygen radicals.

S2: Cardiovascular Performance in Trauma and Sepsis

INSUFFICIENT SPLANCHNIC OXYGENATION CAUSES DEPRESSED CARDIAC FUNCTION **22**

U.HAGLUND, Department of Surgery, Uppsala University, S-751 85 Uppsala, Sweden.

The splanchnic area may enter a situation of flow dependent hypoxia in shock, especially in septic shock. This means that oxygen delivery becomes insufficient relative to the tissue needs. Flow dependent hypoxia is, for one or more of the following three reasons, particularly likely to occur in the splanchnic organs even if the total body oxygen delivery seems adequate: 1. Shock is often followed by a disproportionate flow reduction in the splanchnic area, 2. Changes in flow distribution in shock, as well as the vascular arrangement within the gut wall, further deteriorates mucosal oxygenation. 3. The need for oxygen in the splanchnic area is increased in sepsis. As a consequence, a characteristic superficial mucosal injury develops within 1 to 2 hours of shock. In animal experiments utilizing regional intestinal ischemia or septic shock models the appearance of such mucosal injury has been related to a rather rapid development of cardiovascular derangement. The negative hemodynamic effects were exacerbated with more severe mucosal injury. A comparision between animals with similar degree of mucosal injury revealed that the cardiodepressant effect was more pronounced the larger amount of intestinal tissue made ischemic. Further studies have revealed that the cardiovascular consequences following splanchnic ischemia can be prevented if the development of mucosal injury was prevented by extra local supply of oxygen to the superficial intestinal mucosa. The cause of the cardiovascular derangement seen in this situation was demonstrated to be release of humoral factor(s) to the intestinal venous blood. A water soluble fraction with a molecular mass between 500-1000 Daltons and a lipid soluble fraction with a molecular mass above 10,000 Daltons have been described.

ISOLATION OF A CARDIODEPRESSANT FACTOR PRESENT IN ELEVATED CONCENTRATIONS IN SHOCK PLASMA. S. Hallström, W. Fürst, C. Vogl and G. Schlag. Ludwig Boltzmann Inst. for Exp. and Clin. Traumatology, Vienna, Austria **23**

Controversial results have been publsihed regarding the role and occurrence of myocardial depressant factors in various forms of circulatory shock. We have studied the net inotropic plasma activity in prolonged canine hypovolemic traumatic shock. We found an overall net depression of the low-molecular-weight plasma fraction (ultrafiltrates: mol wt ≤ 1,000) after prolonged hypotension on guinea pig papillary muscle performance in vitro (extent of shortening: -50.6 ± 10.0 % post re-infusion; control: -6 ± 2.6 %, P < .01). Ultrafiltrate-derived plasma compounds were further separated by means of gel permeation chromatography. Inotropic activity of chromatographic fractions, as checked on the papillary muscle assay, led to the identification of a cardiodepressant factor (CDF) elevated 3 to 4-fold in shock plasma (first step: chromatography on Bio-Gel P-2, mobile phase 50 mM NaCl). Salt-free purification of CDF was accomplished in the second step (chromatography on TSK HW 40

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(F)). CDF exerts a dose-dependent negative inotropic effect on the papillary muscle. Inactivation of CDF was achieved with acid hydrolysis and with the specific protease subtilisin, indicating its peptide nature. Further purification (HPLC cation exchange column) of CDF yielded a biologically active fraction containing a hydrophilic peptide (acid hydrolysis: 4 amino acids).

Conclusion: CDF (pathophys. conc.) may contribute to the net negative ino-tropic plasma activity found in the late phase of circulatory shock. The presence of CDF in control plasma and especially its reported mechanism of action (c.f. abstract by Koidl et al., this conference) support the hypothesis that CDF could play a more general physiological role.

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INHIBITORY EFFECTS OF A CARDIODEPRESSANT FACTOR ON EXCITATION AND CONTRACTION OF ISOLATED VENTRICULAR CARDIOMYOCYTES FROM GUINEA PIG.
B.Koidl*, S.Hallström**, U.Müller***, K.Werdan***, and G.Schlag**
*Inst. Med. Physics and Biophysics, Univ. Graz, Austria; **Ludwig Boltzmann Inst. Exp. Clin. Traumatol., Vienna, Austria; ***Med. Dept.I, Univ. Munich, FRG.

By means of photometric measurement of cell pulsation and patch-electrode recording of membrane potential and whole-cell membrane-current, the influence of a cardiodepressant factor (CDF) isolated from the plasma of dogs post hypovolemic-traumatic shock (c.f. abstract by Hallström et al., this conference, for isolation procedures) on excitation and contraction of guinea pig ventricular cardiomyocytes was studied.
CDF reduced the amplitude of contraction in electrically stimulated cells (1 Hz) in a dose dependent manner even at a pathophysiologically relevant concentration of 1.84 ml shock plasma aliquot/ml (substance loss during isolation not considered). This effect could be rapidly reversed by wash-out and could be abolished within seconds by elevation of the calcium concentration in the bath from 1.8 to 10 mmol/l. In the action potentials, an identical dose of CDF resulted in a distinct reduction of the plateau phase and an increase in the duration at 50 and 90 percent of repolarization. Voltage clamp measurements revealed a pronounced inhibition of the calcium current and also a reduction of the delayed rectifier component I_K of the potassium current.
The negative inotropic effect as well as the change of the time course of the action potential can be explained by the observed changes in the ionic currents. The rapid abolition of the negative inotropic effect by elevation of calcium indicates a similar point of attack of CDF and calcium antagonists. The rapid reversibility of the effect by washout, however, suggests a different mode of binding of the substance.

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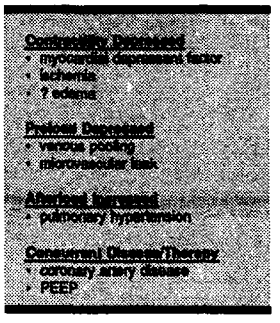
MYOCARDIAL PERFORMANCE IN SEPSIS. W. Sibbald, Program in Critical Care, University of Western Ontario.

The process of *metabolic autoregulation* defines an integrated response at all of the central, regional and microregional levels of the circulation which serves to maintain tissue O_2 availability at appropriate levels. Since the *sepsis syndrome* is characterized by increased tissue O_2 needs, an augmented cardiac output should be anticipated to support an increase in tissue O_2 availability. However, a number of issues contribute to an augmentation of myocardial performance in this syndrome.

Factors contributing to depressed myocardial performance are listed in the Table. It is generally agreed that *Biventricular Contractility* is depressed in the sepsis syndrome, and circulating myocardial depressant factor(s) remain the most likely cause. Other factors which may contribute to depressed biventricular contractility include: (i) ischemia due to dysregulation of myocardial O_2 flux at the microregional level; and, (ii) myocardial edema resulting from the systemic microvascular defect of sepsis.



Depressed Biventricular Preloads remain the most common cause of depressed myocardial performance in the sepsis syndrome, although this problem is easily managed with aggressive fluid resuscitation. Venous pooling in the splanchnic circulation and loss of intravascular volume by virtue of the systemic microvascular defect of sepsis are the more common causes of depressed intravascular volume, and thereby depressed preload. An *Augmented Afterload* usually affects myocardial performance usually only in the right ventricle. In the presence of depressed contractile reserve, an increase in right ventricular afterload complicating pulmonary hypertension may cause pump failure, thereby reducing volume flow from right to left ventricles. Finally, *Concurrent Disease and/or Therapy* may also contribute to myocardial dysfunction in sepsis. Given that sepsis is frequent in the elderly, it must be anticipated that associated coronary artery disease will further reduce myocardial contractile and preload reserve in this syndrome. Finally, it must be remembered that positive pressure ventilation has the capacity to further depress myocardial performance in sepsis.

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EVALUATION OF MYOCARDIAL PERFORMANCE IN HYPOVOLEMIC-TRAUMATIC AND SEPTIC SHOCK. P. Krösl, J.P. Pretorius, R. Hopf, Z. Khakpour, K. Kropik, M. Thurnher, C. Vogl and G.Schlag. Ludwig Boltzmannn Institute for Experimental and Clinical Traumatology, A-1200 Vienna, Austria
Evaluation of myocardial performance under varying loading conditions has always been a challenging task. The most commonly used parameter, i.e. left ventricular (dP/dt)max, is known as a highly sensitive "contractility" index, while at the same time this parameter also varies according to the loading conditions.
We demonstrate the experimental conditions that allow to employ (dP/dt)max and related functions such as the (dP/dt)max-EDD (=enddiastolic dimension)

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relation. With the (dp/dt)max-EDD-relation it is even possible to detect regulation-dependent contractility changes during short-term loading variation (e.g. by occluders placed around the vena cava or aorta). We show that the assessment of myocardial performance with volume-pressure loops, developed by Sagawa for the isolated "balloon" ventricle, has a number of limitations when used in the intact animal. To substantiate his theoretical assumption, Sagawa used constant contractility during volume variation in the isolated ventricle. This, however, does not apply to intact animals. Variations of pre- and afterload by vessel occluders in most instances reveal a non-linear enddiastolic dimension-pressure relation (ESPDR). Thus, the calculation of slope and diameter-axis interceptions produces arbitrary data. We report on several attempts we have made to use other regression functions and to define a characteristic "single-number" parameter (e.g interceptions with diameter-axis or 80 mmHg line).

THE EFFECT OF ACUTE BACTERAEMIC SHOCK ON LEFT VENTRICULAR PRESSURE-DIMENSION RELATIONS IN AWAKE SHEEP. J.P. Pretorius*, P. Krösl, C. Vogl, M. Thurnher, R. Hopf, K. Kropik, Z. Khakpour, H. Redl, G. Schlag.
Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, A-1200, Vienna, Austria.

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The aim of this study was to determine how the left ventricular end-systolic pressure-dimension relation could be used to assess contractile function during acute bacteraemic shock in awake sheep. E. Coli (5×10^7 live colony forming units per kg) was administered as a continuous infusion over 8 hours to 10 sheep. The LV pressure-dimension relation was monitored using a Konigsberg transducer in the LV and one pair of 3 MHz ultrasonic crystals to obtain the external short axis diameter of the LV. By transiently occluding the inferior vena cava and the aortic arch with inflatable occluders, variably loaded pressure-dimension loops were recorded. Calculation of the slopes of the LV end-systolic pressure-dimension relation revealed reflex dependences during partial Aortic occlusion, measurement variability, curvilinearity, unpredictable pressure and dimension changes and inconsistencies in devinding the end-systolic point. Nevertheless, by plotting all end-systolic pressure-dimension data we could show a definite movement into either the positive inotropic or the negative inotropic region during the progression of an eight hour study. Plotting maximum LV dP/dt versus the LV end-diastolic dimension allows the same observation. Using the LV end-systolic pressure-dimension relation and the LV (dP/dt) max- end-diastolic relation as parameters of contractility in the intact cardiovascular system of our shock model in awake sheep, seems to be a valid and more practical alternative than calculating the slope of the end-systolic pressure-dimension relation. Contractility seems to decrease from as early as the fourth hour after initiating an acute bacteraemia.

CARDIAC CONTRACTILE INJURY AFTER INTESTINAL ISCHEMIA-REPERFUSION. J.W. Horton, D.J. White*. University of Texas Southwestern Medical Center, Dallas, TX 75235-9031

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Experimental and clinical data suggest that even a brief period of intestinal ischemia initiates a sequence of events including release of inflammatory mediators and multiorgan failure. This study examined effects of intestinal ischemia and reperfusion on cardiac function in a rat model of the superior mesenteric artery (SMA) occlusion (atraumatic clip for twenty minutes) and collateral arcade ligation. Controls were sham operated (Group 1, N=12). Rats with SMA occlusion were sacrificed at intervals after reperfusion (Group 2, 2-3 hrs, N=10; Group 3, 4-5 hrs, N=10; Group 4, 12-16 hrs, N=10) and hearts were harvested to assess contractile function. In Group 5 (N=12), rats were treated with enteral allopurinol (10 mg/kg/day) for four days before the intestinal ischemia episode; these rats were studied 2-3 hours after reperfusion. There was no change in blood pressure or hematocrit with intestinal ischemia or reperfusion. Cardiac contractile depression occurred as early as two hours after ischemia-reperfusion as indicated by a fall in left ventricular pressure (from 77±3 to 63±4 mmHg, p=0.01) and +dP/dt max (from 1827±59 to 1557±99 mmHg/sec, p<0.02) and -dP/dt max (from 1267±57 to 953±67 mmHg/sec, p=0.02). Contractile depression after ischemia-reperfusion was further documented by a rightward shift in LV function curves and a decreased responsiveness to increases in perfusate Ca^{2+}. Cardiac defects persisted for 16 hours after reperfusion. Allopurinol pretreatment prevented ischemia-reperfusion mediated cardiac dysfunction, and LV function curves calculated for this group were identical to those generated by control hearts. We concluded that 1) intestinal ischemia-reperfusion produces a significant, persistent cardiac dysfunction 2) the cardioprotective effects of allopurinol treatment before ischemia-reperfusion indicate that oxygen derived free radicals contribute, in part, to the cardiac defects.

THE USE OF TRANSCONJUNCTIVAL OXIMETRY TO DETERMINE RESUSCITATIVE ENDPOINT IN THE ACUTELY TRAUMATIZED PATIENT N. Whatley*, J. Wagner*, A. Morgan, E. Hirvela St. Francis Hospital & Medical Center, Hartford, CT 06105

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Pulse, blood pressure, and urine output have traditionally been used to determine adequacy of

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volume resuscitation in hypovolemic shock. However, perfusion at the tissue level may be severely impaired before hemodynamic decompensation is clinically evident. Changes in acid-base status, base deficit, and serum lactate levels more precisely reflect inadequate tissue perfusion. But because such measurements require laboratory assays, results are not instantaneously available. The newly developed transconjunctival oximeter continuously measures conjunctival oxygen tension. Conjunctival oxygen tension has been demonstrated to correlate well with tissue oxygen delivery as measured by invasive monitoring. This easily placed, non-invasive device offers the potential for obtaining a continuous readout of tissue oxygenation in the resuscitative setting. To test the hypothesis that the transconjunctival oxymeter could provide, in a continuous fashion, data more commonly available only via lengthier assays for acid-base status, the oximeter was placed on patients who had sustained major blunt or penetrating trauma. Simultaneous measurements of pcj02 (conjunctival oxygen tension), HR, RR, BP, urine output, ABG, base deficit, serum bicarbonate, and IV replacement were performed. Results from the six patients studied to date demonstrate that the transconjunctival oxymeter is superior to conventional hemodynamic measurements in detecting tissue hypoperfusion. By linear regression analysis there is a highly significant correlation between serum bicarbonate levels and pcj02 (R=.76; p=.006) as well as base deficit and pcj02 (R=.73; p=.007). Thus, the more precise measures of tissue oxygenation previously available only by time consuming laboratory assay are quite accurately reflected via continuous real time readout on the transconjunctival oximeter. We conclude that this new, non-invasive monitoring device will provide an extremely precise and immediate means of measuring resuscitative endpoints in the acute trauma setting.

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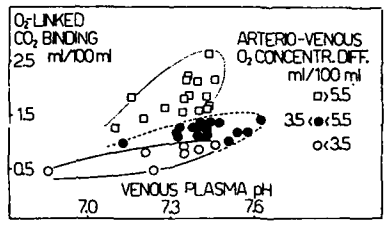
CENTRAL VENOUS CO_2 TENSION ($PvCO_2$) AS A CUMULATIVE INDEX OF CARDIORESPIRATORY AND METABOLIC IMPAIRMENT IN TRAUMA AND SHOCK. C.Chiarla*, I.Giovannini, G.Boldrini*,C.Iannace*, M.Castagneto* Shock Center CNR,Dept Surgery Catholic Univ(F.Crucitti) 00168 Rome, Italy

$PvCO_2$ changes were assessed in 520 measurements performed in 170 pts with critical illness (trauma, hemorrhage, organ failure, sepsis) and in experimental shock. CO_2 production (VCO_2, ml/min/m^2), cardiac index (CI, L/min/m^2), blood respiratory exchange ratio (RE) and base excess (BE), O_2-linked CO_2 binding (O_2-CO_2B) and other variables were determined. In all groups, at CI>2, up to 90% of $PvCO_2$ changes were explained by arterial CO_2 tension ($PaCO_2$)($r^2 \approx .9$, $p<.001$ in all groups). At CI<2, the variability explained by $PaCO_2$ fell to 10%, while worsening CI and venous pH (or BE), and VCO_2 changes, became important. $PaCO_2$, VCO_2, CI and pHv controlled nearly all of $PvCO_2$ variability:

Clinical data: $PvCO_2 = 1.07(PaCO_2)+0.17(VCO_2/CI)-7.15(pHv)+50.14$ $r^2=.98$ $p<<.01$

Experim. data: $PvCO_2 = 1.00(PaCO_2)+0.13(VCO_2/CI)-36.21(pHv)+263.96$ $r^2=.93$ $p<<.01$

At very low CI, discrepancies of RE and pH-mediated falls in O_2-CO_2B (figure) concurred to further explain rises in $PvCO_2$ ($p<.01$ for both). In hyper/normodynamic states high $PvCO_2$ mostly reflects respiratory impairment; in hypodynamic states, $PvCO_2$ cumulatively reflects also hemodynamic failure with high CO_2 transport per unit blood flow, failure of CO_2 binding in blood from acidosis, and the effect of processes with altered RE.



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ANOXIA AND REOXIGENATION IN CULTURED NEONATAL RAT HEART CELLS: A MODEL FOR STUDYING "ISCHEMIA AND REPERFUSION"-INDUCED IMPAIRMENT IN CATECHOLAMINE-MEDIATED INOTROPY?

Boekstegers P.*, A. Pfeifer*, K. Werdan
Dept. of Internal Medicine I, Klinikum Großhadern, University of Munich, FRG

In cultured neonatal rat heart cells anoxia (pO_2<1mmHg) and substrate free media resulted in loss of spontaneous beating activity within 60-90 minutes. Contractility (Vmax, cell wall motion) of electrically stimulated cells (n=44) declined to 30% of the initial value with ATP-content reduced to 30-50%. Reoxygenation using cell culture media restored spontaneous beating activity within 30 to 90 sec.. However, complete recovery of beating activity to preanoxic values lasted 60-90 min.. After reoxygenation contractility had an overshoot reaction of Vmax (110-180% of the preanoxic value) within the first 15 min. of reoxigenation with progressive decline of contractility during the following 45 min. to 80% of the preanoxic value. Repeated periods of anoxia and reoxigenation resulted in loss of overshoot reaction and in a further decrease in contractility after 1 h of reoxigenation. Studying the response to stimulation of the ß-adrenorezeptor-adenylatcyclase system, the cells were perfused with isoprenaline (10^{-5} M) and Ca^{++}(2,4 mM) solution before anoxia, after 60 min. of anoxia and 60 min. after reoxigenation. Before anoxia isoprenaline increased Vmax from 62 to 86% (SD=8.1, n=14) of maximal Ca^{++} effect, during anoxia from 56 to 72% (SD=11). However, after 1 h of reoxygenation isoprenaline did not increase Vmax any more whereas maximal Ca^{++} effect was unchanged. Conclusions: Recovery of contractility after anoxia and reoxygenation in cultured neonatal rat heart cells was quite similar to whole heart preparations exposed to brief periods of ischemia and reperfusion. Particularly, functional response of the ß-adrenoceptor-adenylatcyclase system to pharmacological stimulation can be tested in isolated heart cells under conditions similar to ischemia and reperfusion in order to study mechanisms related to reduced sensitivity of reperfused myocardi m to catecholamines.

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IMPAIRMENT OF THE CONTRACTILE STATE OF CARDIOMYOCYTES BY PSEUDOMONAS EXOTOXIN A

Ursula Müller*, Alexander Pfeifer*, Renate Reng*, Heinz Rupp[1]*, Karl Werdan
Department of Medicine I, Klinikum Großhadern, University of Munich; Department of Physiology II[1], University of Tübingen

Pseudomonas (Ps) Exotoxin A (PsExoA) is a pertinent virulence factor in Ps septicemia. In order to investigate into mechanisms of cardiac impairment in Ps sepsis, spontaneously beating isolated heart muscle cells of neonatal rats were cultured in media containing PsExoA for up to 5 days. To determine the rate of protein synthesis in these cells, ^{3}H-leucine-incorporation (^{3}H-li) was measured for periods of 2h. Contraction amplitude and velocity were monitored by means of a photoelectrical device. Cytotoxicity was determined by morphological criteria and by measuring LDH-release, potassium- and protein-contents of the cells. PsExoA inhibits ^{3}H-li into neonatal rat heart muscle cells (EC_{50} = 10 ng/ml). Maximum inhibition of ^{3}H-li is accomplished after ≤24h for several toxin concentrations tested in the range of 1 to 50 ng/ml. Inhibition of ^{3}H-li is reversible within 48h after washout of PsExoA and can be prevented by the addition of Ps immunoglobulin (1 mg/ml) either given simultaneously or even applied 24h after the toxin. This toxin also impairs recovery from catecholamine desensitization: High catecholamine concentrations, eg. 10^{-7}M noradrenaline, rapidly attenuate the positive inotropic effect of catecholamines, this desensitization being completely reversible 24 h after washout of noradrenaline. However, in the presence of 1 ng/ml PsExoA - resulting in a partial inhibition of protein synthesis of about 20% - the recovery from catecholamine-desensitization is completely abolished, the cells remaining refractory to the positive inotropic effect of isoproterenol. Within the same range of toxin concentrations, the isoproterenol-induced shift in the myosin-isoenzyme population from V_3 to V_1 is suppressed in these cells. In contrast, the same concentrations of PsExoA do not impair the acute inotropic responsiveness of the cells to catecholamines, nor do they bring about significant cytotoxicity.

Conclusions: (1) The protective effect of Ps immunoglobulin in these cells can be explained by reversal of PsExoA-induced inhibition of biosynthesis. (2) Partial inhibition of protein synthesis by PsExoA of about 20% does not grossly impair viability and is without conspicuous effects on spontaneous beating of the cells within several days. (3) However, this partial inhibition of protein biosynthesis by PsExoA impairs recovery from catecholamine-desensitization, which could conceivably be a mechanism of cardiodepression by this toxin.

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NEUROLOGICAL FUNCTION AND RECOVERY IN PROFOUND HEMORRHAGIC SHOCK (HS) IN MONKEYS.

G. Bar-Joseph*, P. Safar, A. Radovsky*, W.S.Stezoski*, H. Alexander*.
International Resuscitation Research Center, Univ. of Pittsburgh, Pittsburgh, PA 15260

Though there is no clinical evidence that survivors of shock suffer any permanent neurological damage, histological abnormalities were demonstrated in arterial boundary zones in animals. We correlated functional and electroencephalographic (EEG) activity and longterm outcome with histology findings in monkeys subjected to profound, prolonged HS. Methods: All observations were made during the development of a new HS model (1) and in studies comparing resuscitation therapies. Cynomolgous monkeys were maintained during HS and early resuscitation on N2O:O2=75:25% analgesia only, breathing spontaneously. In series I they were bled 27 ml/Kg in 20 min. with no further intervention until natural death (n=7) or until fluid resuscitation was started at varying time intervals (n=13). In series II (n=31), after the first 30 min of "natural response", MAP was controlled at 30 mmHg for additional 90 min, when therapy was started. EEG was recorded via 2 fronto-parietal screw electrodes, and was graded on a 0-5 scale (1). Surviving animals were observed for 4-7 days. Results: CNS depression was the presumed cause of early death in 7 of 11 monkeys which died during HS or immediately after start of fluid therapy in series II. Deterioration to coma started at MAP<35 mmHg, disappearance of brainstem reflexes and severe depression of EEG only at MAP<30 mmHg. When MAP was controlled at 30 mmHg, level of consciousness clearly deteriorated with time, while EEG activity remained unchanged. All longterm survivors were neurologically intact 4-7 days after HS. Histological abnormalities were very mild, diffuse and variable, with no predilection to the arterial boundary zones. Conclusions: During HS brain perfusion probably determines survival. Severe functional CNS depression is closely correlated to the level of hypotension but is completely reversible with therapy. Histological damage is minimal and does not seem to have any clinical significance.

1. G. Bar-Joseph et al. Resuscitation 17 (1989) 11-32.

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SIMULATED LUNG INJURY COMPROMISES THE HEMODYNAMIC RESPONSE TO HEMORRHAGE IN THE DOG.

David A. Allen* and Eric R. Schertel* (Spon: G.C. Kramer).
The Ohio State University, Columbus, OH 43210

The association between lung injury and cardiovascular compromise has been well established in the clinical and experimental literature, however the mechanisms responsible are not well defined. Stimulation of lung non-myelinated vagal afferent nerve receptors (pulmonary C-fibers) causes transient bradycardia, hypotension and decreased contractility. These fibers are stimulated and reflexes are elicited by factors present during lung injury; e.g. lung congestion, pulmonary embolization, and inflammatory mediators. We isolated and separately perfused the left pulmonary circulation with an extracorporeal circuit in six chloralose anesthetized, open-chest, atropinized dogs. The right and left airways were isolated with an endobronchial tube and ventilated at a constant rate with 100% O_2 and 95% O_2:5% CO_2, respectively. We used a mild hemorrhage (10% blood volume over 3 min) to evaluate the hemodynamic response in each dog during 1) control conditions, 2) isolated simulated lung injury and 3) isolated simulated lung injury with left pulmonary denervation. Capsaicin, a specific C-fiber stimulant in the dog, was used as the agent simulating lung injury and was infused continuously during hemorrhage at a rate (6.0 +/- 0.98 ug/kg/min) that produced mild pre-hemorrhage decreases in mean arterial pressure (<12%) and cardiac output (<18%). The hemorrhage was performed 3 min after a response was observed. The pre-hem MAP after capsaicin infusion did not differ from control pre-hem MAP. The change in MAP from pre-hem to 1 and 3 min post-hem was significantly greater during capsaicin infusion and the average MAP were significantly lower during capsaicin infusion at 1 and 3 min post-hem than respective control values. Pre-hem CO of capsaicin

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and control did not differ. CO during capsaicin infusion was significantly lower at 1 min post-hem, but not at 3 min. Heart rate did not change significantly. Pulmonary denervation attenuated the effects of capsaicin. We conclude from this preliminary study that isolated pulmonary c-fiber stimulation, simulating lung injury, compromises the hemodynamic response to mild hemorrhage in dogs.

35 CONTRIBUTION OF NEURAL AND HUMORAL FACTORS TO RENAL VASCULAR TONE DURING PROLONGED CANINE HEMORRHAGIC SHOCK.

T.Fujita and S.Koyama
Shinshu Univ. Sch. Med., Dept. Physiol. Div. 2, Matsumoto, Nagano 390, Japan

This study was designed to evaluate roles of renal sympathetic nerve activity and adrenal cathecholamines on renal vascular adjustments during prolonged hemorrhagic shock in anesthetized dogs. Renal nerve activity (RNA) was measured simultanously with arterial blood pressure. The left kidney was perfused at a constant perfusion flow, and renal perfusion pressure (RPP) was measured to assess renal vascular resistance. In animals with intact baroreceptors, hemorrhagic hypotension of 40 mmHg caused to increase RNA to 267 ± 22 % of the control level one min after bleeding. There followed a recovery to the control level within 10 min after bleeding. Thereafter, a secondary increase in RNA occurred so that 50 min after bleeding RNA was 304 ± 67 % of the control level, followed by a gradual decline toward the control level when the time passes. RPP showed a progressive and significant increase until the end of the experiment. The initial increase in RNA and RPP within 10 min after bleeding was abolished by deafferentation of both carotid sinus and vagal nerves. Increase in RPP at the late stage was abolished after bilateral adrenalectomy. These results indicate that an early renal vasoconstriction during hemorrhagic shock is regulated by reflex sympathetic mechanism, and late vasoconstriction is mediated by humoral substances such as adrenal catecholamines which releases are triggered by reflex sympathetic activation.

36 DIFFERING CONTROL OF SYMPATHETIC ACTIVITIES ON VARIOUS ORGANS DURING HEMORRHAGIC HYPOTENSION IN DOGS.

SHOZO KOYAMA
Shinshu Univ. School of Med. Dept. of Physiol. Division 2 Asahi, Matsumoto, Nagano 390, Japan

These experiments were designed to investigate whether hemorrhagic hypotension of 50 mmHg for 10 min in dogs produces differential control of sympathetic nerve activities to various organs (heart, kidney, liver, spleen and adrenal gland). All of measured nerve activities increased significantly. There followed by a gradual decline of all nerve activities so that 10 min postbleeding only RNA showed -16 ± 19% below the control. In cervical vagotomized dogs, sympathoinhibition in RNA below the control during hypotension was reversed to significant increases after cervical vagotomy. In contrast, animals with carotid sinus denervation alone showed initial increases in nerve activities followed by a recovery to the control within 2 min after bleeding. Following complete denervation of systemic baroreceptors, rapid hemorrhage did not cause any significant change in sympathetic nerve activity in each nerve. These results indicate that early reflex responses to hemorrhage on regional sympathetic nerves are controlled with a summative manner between unidirectional sympathoexcitation by unloading of arterial baroreceptors and the cardiopulmonary receptors. When the time passes after bleeding over one minute, reflex sympathoinhibitiory mechanism which is mediated through the vagal afferents from cardiopulmonary receptors participates to inhibit the unidirectional sympathoexcitation mediated by arterial baroreceptors.

37 EFFECT OF CARDIAC TAMPONADE-INDUCED HYPOTENSION ON SYMPATHETIC OUTFLOW TO KIDNEY, HEART, ADRENAL GLAND, AND LIVER.

T.Shibamoto, Y.Matsuda and S.Koyama.
Shinshu Univ. Sch. Med.,Dept. Physiol., Div.2, Matsumoto 390, Japan.

The purpose of this study was to determine in anesthetized dogs if sympathetic hypotension produces differential changes in efferent sympathetic nerve activity of kidney (RNA), heart (CNA), adrenal gland (ANA) and liver (HNA) and to define how the baroreceptor reflex modulates these responses. We recorded RNA, CNA, ANA, and HNA simultaneously in each animal during cardiac tamponade-induced sustained hypotension to mean arterial pressure (BP) of 50 mmHg for 10 min. Each sympathetic nerve activity in dogs with baroreceptor intact increased and reached to peak level when BP fell to 50 mmHg. Thereafter, RNA decreased to 72±12% of the

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control level and heart rate (HR) decreased from 180±4 to 160±9 beats/min during sustained hypotension, but CNA, ANA and HNA were elevated above the control level. Decreases in RNA and HR during sustained hypotension were reversed by cervical vagotomy to 143± 16% and 189±8 beats/min, respectively. In contrast, increases in CNA, ANA and HNA were not affected by vagotomy. However, the combined vagotomy and sinoaortic denervation abated these responces. Thus, we conclude that hypotension due to cardiac tamponade *in dogs produces directionally* different sympathetic outflow to the kidney, heart, adrenal gland and liver with sympathoinhibition in kidney and sympathoexcitation in other organs, and that the renal sympathoinhibition are mediated by vagal afferents.

THE ROLE OF EICOSANOIDS IN CNS IN THE PATHOGENESIS OF LIMB ISCHEMIA-INDUCED TRAUMATIC SHOCK. Ibolja Cernak, J.Savic (Spon.: J.Savic)
Inst. for Med. Research, Military Med. Academy, Belgrade, 11000 **38**

It was established that traumatic shock, with the same degree of hypovolemia, has much worse outcome than hemorrhagic shock. We postulated that disturbances of cellular energy metabolism in the brain due to hypovolemia, in traumatic shock are aggravated by neural impulses originating from the injured region. Since eicosanoids in the brain have an important role in the regulation of cerebral blood flow, we assumed that the outcome in traumatic shock is significantly dependent on the relationship between brain vasoconstrictive (peptidoleukotrienes, TxA_2, $PGF_{2\alpha}$) and cerebroprotective eicosanoids (PGD_2, PGI_2). The levels of eicosanoids were measured in the rat brain (cortex, thalamic region), subjected to tourniquet trauma, immediately, 30 or 60 minutes after tourniquet removal. Our results indicate that increase of peptidoleukotrienes, which is correlated with cerebral edema, and decrease of cerebroprotective eicosanoids are closely associated with the course of traumatic shock. That the brain eicosanoid changes were causally related to the afferent neural impulses was supported by results obtained following attenuation (spinal anesthesia) or amplification (transcutaneous diffuse electrical stimulation of leg) of afferent neural impulses.

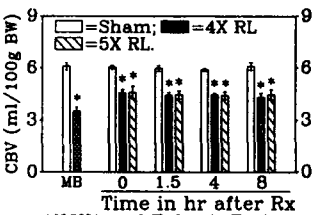
MICROCIRCULATORY AND CELLULAR DISTURBANCES IN HEMORRHAGIC SHOCK RABBITS. Huiming Jin, Gongming Tao and Youzhen Yan.
Dept. of Pathophysiology, Shanghai Medical Univ., Shanghai 200032, P.R.China **39**

Changes in microcirculation of the bulbar conjunctiva, mesentery and kidney; contents of plasma lactic acid, plasma B-glucuronidase;time for RBC electrophoresis and function of platelet aggregation were observed and measured before and during hemorrhagic shock in rabbits. Renal blood flow distribution before and during hemorrhagic shock was determined by fluorescence angiography. Results showed that microcirculatory disturbances in the three sites were obvious, but not quite similar. The changes in conjunctival blood flow was noticed prior to constriction of conjunctival microvessels; the changes in renal surface microcirculation occur in reverse order as compared with those seen in conjunctiva; more obviously abnormal changes in the blood state than in the microvessels were seen in the mesentery. During late stage of hemorrhagic shock the contents of lactic acid and B-glucuronidase increased, the time for RBC electrophresis was prolonged and the platelet aggregation rate decreased. It might be believed that the decrease in charge of RBC is related to the abnormal blood state and that the increase in lactic acid and B-glucuronidase, the decrease in platelet aggregation rate are the results of the disturbances of microcirculation. Authors suggest that the cellular disturbance is followed by microcirculatory disturbance in hemorrhagic shock.

MEASUREMENT OF CIRCULATING BLOOD VOLUME (CBV) IS AN ESSENTIAL PARAMETER FOR DETERMINING THE ADEQUACY OF FLUID RESUSCITATION (Rx) AFTER HEMORRHAGE. P. Wang, Z.F. Ba*, A. Ayala, J.M. Harkema and I.H. Chaudry. Dept. of Surg., Michigan State Univ., E. Lansing, MI 48824 **40**

Although parameters such as cardiac output (CO), mean arterial pressure (MAP), and central venous pressure (CVP) are routinely used to assess the adequacy of fluid Rx after trauma and hemorrhage, it is not known if there is any correlation between restoration of CO and CBV. To study this, rats (~300g) underwent a midline laparotomy (i.e., trauma induced) and were bled to and maintained at a MAP of 40 mmHg until 40% of maximum bleedout (MB) volume was returned as Ringer's lactate (RL). They were then resuscitated with 4 or 5 times (X) the volume of MB with RL. CBV was measured at various intervals. Indocyanine green (ICG, 0.1 mg/rat) was administered intravenously and its clearance *in vivo* was measured without blood sampling by using a fiberoptic catheter and *in vivo* hemoreflectometer (IVH). Initial concentration of ICG ($[ICG]_0$) was calculated from [ICG] versus time (t) plot according to [ICG] =e^(a+bt+ct^2), where e^a is $[ICG]_0$. CBV was determined by ICG dose/$[ICG]_0$. CO was measured with ICG dilution technique by using IVH.

Notes



Results (mean±SE; n=7/group) in the Figure indicate that RL Rx significantly improved the decreased CBV but did not restore it to control despite the fact that CO was restored and CVP was more than doubled. Moreover, tissue water content in various organs increased, indicating significant tissue edema. Since hepatocellular function and renal function were impaired even after 5X RL Rx, the lack of restoration of CBV might be responsible for the subsequent cellular dysfunction and organ failure observed after hemorrhage. Thus, the measurement of CBV appears to be an essential parameter for evaluating the adequacy of fluid Rx after severe hemorrhagic shock.

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CARDIOPULMONARY HEMODYNAMIC AND PERIPHERAL CIRCULATORY RESPONSES IN SHOCK

T. Muteki, N. Kaku, T. Fukushige, I. Kohno and T. Hiraki
Department of Anesthesiol,. & CCM, Kurume Univ. School of Med., 67 Asahi-machi, Kurume-shi, Japan

In order to document cardiopulmonary hemodynamic maladjustments in low flow states in the shock syndrome, optimizing oxygen delivery ($\dot{D}o_2$) and consumption ($\dot{V}o_2$), homeostatic prediction in humoral responses in shock under elevated intrathoracic pressure due to controlled ventilation , and maintenance of vascular resistance of venous return (Rvr) and left ventricular preload reserve (LVPR) due to vasoactive substances and resuscitation fluid treatments were investigated. [Subjects and method] 22 mongrel dogs with an acute oleinic acid pulmonary edema and 17 cases of the critically ill patients with elevated PVR, ARDS and septic shock were involved. Comparative studies on the difference of changes in cardiopulmonary hemodynamic parameters due to administration fo vasodilators, PGE_1 and nitroglycerine (GTN) with cross over study of volume therapy under IPPV with PEEP and HFJV. [Results] Interrelationship diagram between $\dot{V}o_2$ and $\dot{D}o_2$ indicates better response in the PGE_1 group. RVWI and Rvr reduced more significantly in PGE_1 group. Mean right coronary artery perfusion pressure was significantly higher maintained in PGE_1 group. The 'x' wave of RaP, PAP, PCWP, and LVEDP significantly decreased and plasma level of α – ANP elevated during HFJV. Skin blood flow of the thigh and postischemic response of capillary BF of the fingertip in RV dysfunction (RVEF ↓) were higher maintained in PGE_1 group. [Conclusion] These data suggest that shock patients, especially those with poorly preserved RVEF can benefit from augmentation of LVPR and reduction of Rvr due to resuscitation fluid and vasodilator (PGE_1) treatment under HFJV with manipulation of the pressure.

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EFFECTS OF THYROTROPIN RELEASING HORMONE (TRH) ON HEMODYNAMICS IN COMATOSE PATIENTS

M. Aibiki, Y. Shirakawa, S. Ogura, K. Honda, O. Umegaki, K. Seki and K. Ogli. Kagawa Med. Sch., Dept. of Anesthesiology & Emergency Med. Kagawa, 761-07, Japan.

(INTRODUCTION) Thyrotropin releasing hormone (TRH) has been indicated to exert hypertensive effects through the central nervous system. Thus, hemodynamic effects of TRH may vary if TRH is administered to patients with an impairment in the central nervous system. We evaluated hemodynamic changes when TRH was administered to counter consciousness disturbances in comatose patients. **(METHODS)** The comatose patients were divided into two groups: the vegetative group, preserving spontaneous respiration and brain stem functions (N=7) and the brain death (BD) group, fitting the Japanese criteria for BD (N=10). In the two groups, changes in blood pressure and heart rate were evaluated when TRH was administered (0.1 mg/kg, iv). Patients who had diabetes mellitus and arrythmias were excluded from this study. **(RESULTS)** In all vegetative patients, TRH caused hypertension with tachycardia. In contrast, no changes in blood pressure and heart rate were found in eight of ten BD patients after TRH injection, the remaining two showing an elevation in blood pressure. **(CONCLUSION)** These results indicate that in comatose patients, the hemodynamic effects of TRH may differ depending on impairments in the central nervous system. Two BD patients, exhibiting hypertensive responses to TRH, showed spinal reflexes. Thus, there is a possibility that these hypertensive effect of TRH may be mediated through TRH receptors in the spinal cord.

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EFFECTS OF AIR EMBOLISM ON HEMODYNAMICS AND RENAL NERVE ACTIVITIES IN URETHANE-ANESTHETIZED RABBITS

S. Ogura, M. Aibiki, K. Honda, O. Umegaki, K. Seki, Y. Shirakawa and K. Ogli. Kagawa Med. Sch. Dept. of Anesthesiology & Emergency Med. Kagawa, 761-07, Japan.

We designed this experiment to evaluate the effects of an intravenous

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bolus injection of air on systemic blood pressure (SBP), heart rate (HR), central venous pressure (CVP) and renal nerve activities (RNA) in rabbits. Animals were divided into the following groups: animals with neuraxis intact (I group, N=6), cervical vagotomized animals (V group, N=5), sino-aortic denervated animals (SAD group, N=5) and sino-aortic denervated animals with cervical vagotomy (SADV group, N=6). Each group was challenged with an intravenous bolus injection of air (0.5 ml/kg) to evaluate effects of air embolism on the measured parameters. In the I group, the injection of air caused profound hypotension associated with a significant increase in CVP. RNA response showed an increase in spite of a drop in HR. In contrast, vagotomized animals did not exhibit a significant decrease in SBP even when there was an increase in CVP and RNA with administration of air. In the SAD group, a rapid and profound decrease in SBP and RNA occurred after injection of air. An increase in CVP was similar to that of the I group. Animals in the SADV group showed significant hypotension with an increase in CVP after administration of air. However, while an initial RNA response did not change significantly, it was followed by a remarkable augmentation in RNA. These results indicate that inputs of vagal afferents may have a role in the development of hypotension induced by a bolus injection of air.

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EFFECT OF INCREMENTAL POSITIVE END-EXPIRATORY PRESSURE(PEEP) VENTILATION ON RIGHT VENTRICULAR(RV) PERFORMANCE AFTER ADEQUATE RESUSCITATION FROM SEPTIC SHOCK. AJ Schneider, ABJ Groeneveld, D deJONG, RIC Wesdorp, LG Thijs. Free University Hospital. PO Box 7057, 1007 MB Amsterdam, The Netherlands,
Objective: to study the effect of a stepwise increase in PEEP on RV volumes and RV ejection fraction(EF)in combination with conventional hemodynamics in 11 patients resuscitated from septic shock.
Design: Patients were studied on the surgical intensive care and were intubated and ventilated with a rate of 15 to 20 per min. Electrocardiogram, arterial pressure, pulmonary arterial pressure, pulmonary wedge pressure, central venous pressure, cardiac index, RV end-diastolic volume(RVEDV), RV end-systolic volume(RVESV) and RVEF were monitored by a radial artery cannule and a Swan Ganz catheter with a rapid transmistor(REF-catheter). Injection of 5 ml. glucose solution(5%) was automatically performed by a phase controller and pneumatically driven syringe after a manual start of the REF-computer. The moment of injection in the ventilatory cycle was derived from a Siemens Servo ventilator which delivers 100 impulses during each ventilatory cycle. The injections were done successively at every 10 percent of the total ventilatory cycle during each PEEP-level(5, 10, 15, 10, and 5 cm H2O). The mean value of 11 measurements was accepted to be the real value.
Results: At 15 cm H2O PEEP cardiac index and mean arterial blood pressure decreased, heart rate remained unchanged. Mean pulmonary arterial pressure, pulmonary wedge pressure and central venous pressure increased. At maximum PEEP RV stroke volume, RVEDV, RVESV fell whereas RVEF did not change significantly. Mean RV stroke volume related linearly and positively with mean RV end-diastolic volume.
Conclusion: incremental PEEP ventilation in patients resuscitated from septic shock induces a fall in RV end-diastolic volume and RV stroke volume. Pump function was unchanged.

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HEAT SHOCK ATTENUATES ENDOTHELIUM-DEPENDENT VASODILATION IN SKELETAL MUSCLE MICROCIRCULATION A.S.Lübbe. Dept.Medicine, Virchow Med.School, 1000 Berlin 19, FRG.

Endothelium-derived relaxing factor (EDRF) mediates vasodilation of small arterioles under various (patho)physiological conditions: E.coli sepsis, systemic hypoxia, topical acetylcholine (ACH). To test, if heat shock (HS) changes EDRF-dependent reactivity arterioles to ACH, we used the in vivo cremaster muscle of anesthetized rats (175 ± 1 g). In group 1 (n =7), diameters of fourth-order arterioles (A_4) were measured to increasing ACH dosages (10^{-7} – 10^{-4} M). In group 2 (n=5), the cremaster temperature was increased to 40°C for 60 min., then reduced to 34°C for 60 min. before ACH was topically applied. In group 3 (n=5), body temperature was increased to 41°C (whole-body hyperthermia, WBH) for 30 min., then reduced to 37°C for 30 min. before ACH was applied. Nitroprusside (NPR) induced maximal vasodilation. Blood pressure (BP) and A_4-data were expressed as % change from baseline (BL ±SEM) (*p<.05 for 2 versus 1 and 3 versus 1).

	BL	experimental design				ACH				NPR
						10^{-7}	10^{-6}	10^{-5}	10^{-4}	
group 1		saline control								
	mm Hg		*30 min*	*60 min*						
	BP	108 ±3	95 ±1	97 ±3						
	A_4	7 µm	98 ±2	102 ±4		142 ±18	184 ±24	222 ±26	241 ±28	244 ±25
group 2		40°C local heat		34°C post HS						
	mm Hg	*30 min*	*60 min*	*30 min*	*60 min*					
	BP	115 ±4	96 ±3	93 ±4	93 ±4	94 ±3				
	A_4	7 µm	93 ±4	99 ±3	109 ±10	101 ±8	118 ±8	121 ±7*	145 ±6*	166 ±8*	216 ±16
group 3		41°C WBH		37°C post WBH						
	mm Hg	*10 min*	*30 min*	*10 min*	*30 min*					
	BP	108 ±4	110 ±4	95 ±10	96 ±6	106 ±6				
	A_4	7 µm	106 ±10	100 ±8	123 ±6	121 ±8	141 ±16	157 ±25	145 ±10*	196 ±26*	224 ±25

Local and whole-body heat shock did not alter blood pressure and diameter of large (not shown) and small arterioles. Both heat protocols reduced reactivity of A_4 to ACH, but not to NPR. Thus, heat shock could change EDRF generation or efficacy in the post-hyperthermia phase in the skeletal muscle microcirculation.

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46 DOWN REGULATION IN MYOCARDIAL BETA-ADRENERGIC RECEPTORS DURING HYPERDYNAMIC ENDOTOXIN SHOCK

K.Okada, M.Yahagi*, T.Honda*, S.Tezuka*, K.Mizumachi* Department of Anesthesiology, Teikyo University School of Medicine, 2-11-1 Kaga Itabashi-Ku, Tokyo 173, Japan

Beta- and Beta$_1$-adrenergic receptor (B-, B_1-AR) binding sites were determined with techniques of radioligand binding study in myocardial membranes from rabbit left ventricles 18 h after intraperitoneal administration of sterile saline (SAL) or E.Coli endotoxin (LPS; 50 μg/kg) (Group E), as well as 3 h after intravenous injection of SAL or cytokines (interleukin 1: IL-1; 5 μg/kg followed by 25 ng/kg/min for 2 h, or tumor necrosis factor: TNF; 5 μg/kg) (Group C). [^{3}H]Dihydroalprenolol ([^{3}H]DHA) was used as radioligand, and the density of B_1-ARs was assessed as the binding of [^{3}H]DHA in the presence of ICI 118,551 (5×10^{-9} M), a highly selective B_2-AR antagonist. In Group E, measurements of mean arterial blood pressure (MAP), heart rate (HR), and cardiac output (CO) (by thermodilution) were made under pentobarbital-anesthesia before left ventricles were removed for membrane preparations. Data are summarized in the following table.

GROUP-INFUSION(n)	MAP (mmHg)	HR ($beat^{-1}$)	CO ($l\cdot min^{-1}$)	B-AR Bmax (fmol/mg protein)	B-AR K_D (nM)	B_1-AR Bmax (fmol/mg protein)	B_1-AR K_D (nM)
E-SAL(6)	100±4	309±13	0.61±0.05	58.9±2.9	8.7±1.0	53.2±2.7	12.2±1.9
E-LPS(6)	94±2	285 16	0.75±0.02*	48.2±4.3°	8.3±1.0	43.7±5.7	11.9±1.3
C-SAL(6)	--	--	--	60.5±3.2	7.2±0.7	54.3±2.9	11.7±0.6
C-IL-1(6)	--	--	--	56.7±3.5	8.3±0.6	49.4±3.5	12.1±1.0
C-TNF(5)	--	--	--	55.3±3.7	8.3±1.1	49.3±2.9	13.4±2.2

Each data represents mean±SEM of duplicate determinations.
*P<0.05 vs E-SAL by Student's t test. °P<0.05 vs E-SAL by Mann-Whitney U test.
Decrease in Bmax in E-LPS group would suggest a down regulation of B-ARs during hyperdynamic endotoxin shock induced by intraperitoneal administration of low dose LPS.

47 CHANGES IN Emax AFTER ADMINISTRATION OF TUMOR NECROSIS FACTOR.

M.Horibe*, S.Tezuka*, and K.Okada.
Department of Anesthesiology, Teikyo University School of Medicine, 2-11-1 Kaga Itabashi-Ku, Tokyo 173, Japan.

Tumor necrosis factor (TNF) is considered to be one of circulatory deteriorating factors during endotoxin shock. Previous studies demonstrated that administration of cytokines was associated with hypotension and hemodynamic changes typical of septic shock. Parrillo assessed the hemodynamic effects of interleukin-2 using continuous hemodynamic monitoring and determinations of left ventricular ejection fractions. In this study, we evaluate the cardiac function before and after intravenous administration of TNF (10 μg/kg) in dogs. Cardiac contractility was estimated by measuring maximal elastance (Emax), which was independent of preload, afterload, and pulse rate. Dogs were anesthetized with pentobarbital, and respiration was controlled to keep arterial PCO_2 about 35 mmHg. A Swan-Ganz catheter was placed in the pulmonary artery and cardiac output was determined by thermodilution method. After thoracotomy, the conductance catheter, the eight-electrode catheter described by Baan, was inserted from the left ventricle apex to just above the aortic valve, and was connected to signal processor (Model Sigma 5, Leycom, The Netherlands). And then, Miller catheter was inserted into the left ventricle. Intraventricular volume changes were continuously determined with conductance catheter. Both volume (X-axis) and pressure (Y-axis) changes in left ventricles were displayed on an oscilloscope. Emax is possible to obtain by changing the size of left ventricle. Inferior vena cavae were clamped to decrease the size of ventricle, and similar measurement of both volume and pressure in the ventricle was repeated. TNF induced slight decreases in blood pressure and cardiac output, and also produced a progressive decrease in Emax. These data suggest that TNF may be a major contributing factor in deteriorating the cardiac function during endotoxin shock.

48 REGULATION OF ADENYLYL CYCLASE BY TUMOR NECROSIS FACTOR ALPHA (TNFa) IN RAT CARDIOMYOCYTES.

C.Reithmann*, P.Gierschik*, K.H.Jakobs* and K.Werdan.
Dept. of Medicine I, Grosshadern Munich Univ. Hospital, D-8000 Munich, F.R.G. and Dept. of Pharmacology, Univ. Heidelberg, D-6900 Heidelberg, F.R.G.

The decrease in catecholamine sensitivity in heart failure and sepsis is mainly attributed to a desensitization of adenylyl cyclase by an increased level of circulating catecholamines. This desensitization of adenylyl cyclase has been reported to be due to a down-regulation of $ß_1$-adrenoceptors ($ß_1$-AR) and an increase in the level of the alpha subunits of the inhibitory G-protein of adenylyl cyclase (G_{ia}). Plasma levels of the cytokine TNFa have also been shown to be increased in heart failure and sepsis. In several cell types TNFa and catecholamines have been reported to produce similar effects such as on the expression of receptors and other membrane proteins. Therefore, - using the experimental model of rat cardiomyocytes - we studied whether TNFa exposure may also alter the level of adenylyl cyclase components and catecholamine sensitivity in the heart. TNFa (10 U/ml) exposure of rat cardiomyocytes for 48 h decreased the level of $ß_1$-AR by about 40 % and increased the level of G_{ia} by about a factor of 2-3. In addition, the level of the G-protein ß-subunit and the activity of the catalytic subunit of adenylyl cyclase which are unaltered by catecholamine treatment were also increased by the TNFa exposure (by about a factor

Notes

of 2-3 and by 50 %, respectively). As a consequence, TNFa treatment did not decrease but even increase adenylyl cyclase activity.
Conclusion: Treatment of rat cardiomyocytes in the presence of TNFa alters the level or activity of several adenylyl cyclase components and increases catecholamine sensitivity. These effects of TNFa may contribute to an increased catecholamine cardiotoxicity in heart failure and sepsis.

S3: Prophylactic Measures in Trauma

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PROPHYLAXIS OF LETHAL PULMONARY COMPLICATIONS BY EARLY INTUBATION AND TUBE-THORACOSTOMY: A COMPARISON OF TRAUMA RESCUE MODALITIES. M.L. Nerlich, M. Holch, W. Stange, M.Muggia-Sullam*. Department of Trauma Surgery, Hannover Medical School, Hannover, Germany, * Department Surgery A, Hadassah-University, Jerusalem, Israel

A population of 6303 accident victims included 570 deaths (D) and 105 survivors (S) with an ISS>20 (mean=37.8). 248 of D and 49 of S had severe chest trauma (AIS>3) Effects of intubation and tube thoracostomy (TT) on outcome were compared between trauma center and physician staffed rescue helicopter (TC) to other hospitals and rescue systems (OH) (Tab1). Intubation was performed either primarily (I-E), for complications (I-C) or was not performed, despite an indication to do so (I-O). TT was performed early (TT-E) delayed (TT-D) or was not performed (TT-O) although indicated (Tab.2).

Tab.1:

	total	D<1h (%)	D>1h<24h (%)	D>24h (%)	S (%)
CT	297	122 (41)	73 (25)	53 (18)	49 (16)
TC	108	---	46 (43)	29 (27)	33 (31)
OH	74	---	34 (46)	24 (33)	16 (21)

Tab.2:

	EMT			emergency MD			emergency room		
Death time	<1d	>1d	S	<1d	<1d	S	< 1d	>1d	S
I-E	15	0	2	67	15	19	1	6	10
I-C	--	-	-	--	--	--	24	17	9
I-O	20	2	0	7	4	1	0	1	0
TT-E	--	-	-	2	3	6	18	11	18
TT-D	--	-	-	--	--	--	2	8	5
TT-O	--	-	-	15	4	0	6	2	0

I and TT were performed more often and earlier by the helicopter physician, and had a beneficial effect on decreasing early and late mortality. Delayed procedures or their omission led to increased early and late mortality.

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FRACTURE MANAGEMENT AND PULMONARY FAILURE. R.J.A. Goris
University Hospital Nijmegen, P.O.Box 9101, 6500 HB NIJMEGEN, The Netherlands.

Historically it was taught that fracture management in multiple trauma should be postponed as at the time of admission the patient was too sick for any additional surgery beyond the immediate life and limb saving surgery. The result was a substantial number of late deaths due to sepsis and MOF. In the seventies immediate internal fixation of femur fractures became the subject of inquire in a few centers. Reports using the ISS showed that immediate fixation of major fractures together with prophylactic mechanical ventilation worked even with severely injured man to reduce drastically the time on the ventilator and to prevent late septic death. Experimentally, internal stabilization of a fractured femur prevented a significant drop in $Pa\,O_2$ in dogs. In ventilated pigs with a combined missile trauma and fractured femur, early osteosynthesis of the femoral fracture prevented respiratory or circulatory failure. The multiple trauma patient benefits greatly from immediate operative fracture stabilization followed by prophylactic mechanical ventilation and early mobilisation. This results in reduced time on the ventilator and in the ICU. It also produces less need of pain medications, a markedly reduced incidence of pulmonary failure, disappearance of late septic deaths, and a considerable reduction in the mortality rate while providing care at a lower total cost.

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PROPHYLACTIC MEASURES: THE VALUE OF EMERGENCY MEDICINE IN THE PREVENTION OF ACUTE LUNG FAILURE, MULTIPLE ORGAN FAILURE AND SEPSIS AFTER POLYTRAUMA. H. Benzer, W. Koller, Ch. Putensen, U. Waibel
Univ. Innsbruck, Department of Anesthesia and Intensive Care Medicine, A-6020 Innsbruck

Multiple Organ Failure and Sepsis are presently the major cause of morbidity and death among polytrauma patients. As prophylactic step to prevent organ failure the following clinical methods are available: 1. Early Ostheosynthesis, 2. Pharmacologic Intervention, 3. Selective Decontamination of the Digestive Tract, 4. Skilled Emergency Medicine in the Prehospital Phase and 5. Early Ventilatory Support.
In a prospective study (152 severe polytraumatized patients, mean ISS 37 points) group 1 (84 patients), admitted within 60 minutes after trauma,

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had 18% ARDS, 43% MOF and 27% sepsis, whereas group 2 (68 patients), admitted via small hospitals, not before 3 hours after trauma in our emergency room, had 35% ARDS, 61% MOF and 43% sepsis.
In group 1 we could start our Strategy of Ventilatory Support (1. Early Ventilatory Support, 2. Correct, ideal dosage of ventialtion already when starting ventilatory support, 3. CMV as short as possible and less invasive as possible) within 60 minutes after trauma, in group 2 not before 3 hours after trauma. Lack of correct ventilatory support shoud be one of the reasons for higher incidence of complications in the patients of group 2.

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PROPHYLACTIC MEASURES: THE USE OF EXOGENOUS SURFACTANT TO ARREST PROGRESSION OF ACUTE LUNG INJURY - THERAPEUTIC CONSIDERATIONS. R.G. Spragg, N. Gilliard, D. Pappert, D. Hite, R.M. Smith. Division of Pulmonary and Critical Care Medicine, Univ. Calif. San Diego, San Diego, USA.

Analysis of bronchoalveolar lavage fluids (BAL) from patients with acute lung injury indicates that reactive oxygen products, neutrophil proteases, and a variety of plasma proteins are present in the distal airway. Exposure of lung surfactant (LS) to Fe^{++} + H_2O_2 results in liberation of lipid peroxidation products and loss of LS surface tension lowering function as determined *in vitro* using the bubble surfactometer or *in vivo* in the surfactant deficient fetal rabbit. Similarly, exposure of LS to neutrophil proteases results in LS apoprotein digestion and may cause loss of LS function. Finally, several plasma proteins have been shown to cause loss of LS function. Analysis of LS in BAL collected from patients with acute lung injury discloses evidence of abnormal chemical structure of LS and of loss of LS surface tension lowering function. This functional loss may result in progressive atelectasis, shunt, and hypoxemia. To prevent this progression and to attempt to restore lung gas exchange function, we have administered porcine surfactant, 50 mg/kg, to 6 patients with acute lung injury. This administration was accompanied by a transient improvement in gas exchange, increased BAL phospholipid content, and improved BAL LS function. To optimize delivery of LS, we have initiated studies to study the effect of dose volume, concentration, and delivery method on LS distribution. Results suggest a direct relationship between dose volume and homogeneity of distribution. Surfactant treatment of patients with acute lung injury remains a promising, albeit experimental, modality.

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PROPHYLACTIC MEASURES TO PREVENT MULTIORGAN FAILURE FOLLOWING ACID ASPIRATION. H. Hechtman, The Brigham and Women`s Hospital and The Harvard Medical School, Boston, MA 02115
Acid aspiration induces a local and systemic inflammatory response which is characterized by neutrophil sequestration and increased microvascular permeability. Two distinct pathways are thought to mediate PMN-endothelial interaction. The first is rapid and consists of early eicosanoid generation leading to neutrophil activation, that is a PMN oxidative burst and functional upregulation of PMN adhesion receptors. The early lung leukosequestration is likely transient and is not accompanied by vascular injury. The second, delayed PMN sequestration is related to protein and cytokine synthesis which mediate endothelial activation. This endothelial activation is associated with a long lived PMN-endothelial interaction and microvascular barrier dysfunction. It is our thesis that only this delayed endothelial activation, leading to strong PMN adhesion to endothelium, leads to neutrophil release of oxygen free radicals and elastase. Inhibition of eicosanoids or cytokines prevents both the PMN and endothelial activation and thereby will block PMN-endothelial interactions. After aspiration there is a potential therapeutic window of 1 to 2 hours. During this time interval, inhibition of neutrophil adhesion using a mAb directed against neutrophil or endothelial adhesion receptors may protect from acid injury. Other possible treatments are those using agents which block eicosanoids, reactive oxygen species or elastase. Inhibition of these mediators is effective even 1 to 2 hours following aspiration. Finally, enhancement of microvascular barrier tightness using agents that assembly the endothelial cytoskeleton will also minimize neutrophil-induced pulmonary edema.

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THE THERAPEUTIC POTENTIAL OF EXOGENOUS SURFACTANTS MIGHT BE RELATED TO A PMN ADHERENCE LOWERING EFFECT. W. Strohmaier, R. Kneidinger, B. Müller*, H. Redl and G. Schlag. Ludwig Boltzmann Inst. for Exp. and Clin. Traumatology, Vienna, Austria; Pulm. Res. Lab., Policlinic, Dept. Int. Med., Marburg, FRG.

We have recently shown that intratracheal administration of natural (NS),

natural modified (NMS) and synthetic surfactant (SS) reverses lung mechanic properties in a rabbit aspiration trauma model. It was also shown that NS has by far the highest therapeutic potential with regard to effect and dose. Because leukocyte (PMNL) adhesion to the alveolar epithelium is the initiating event in mediating cell damage we investigated whether surfactant has an influence on PMNL adhesion in vitro and if there are differences due to surfactant composition. Freshly isolated human PMNL were stimulated with FMLP. After 30 min either NS, SS (DPPC: Egg PG 4:1), rat surfactant protein A (rSP-A) or the combination SS + rSP-A (9:1 w/w) was added. After additional 30 min. adherence to plastic was measured photometrically via myeloperoxidase.

NS°	SS°	rSP-A	SS + rSP-A°
50.8±12.3	69.5±8.3	74.5±19.8	76.1±8.0

Data are presented as mean (± SD) percentage of FMLP stimulated PMNL adherence (= 100 %), °$p < 0.01$ compared to stimulated PMNL alone. All surfactant preparations significantly lowered PMNL adherence in vitro. rSP-A alone had no effect. SS with and without rSP-A showed similar effects. Whether the more pronounced influence of NS - in vivo and in vitro - is due to the interaction of natural phospholipids and SP-A deserves further elucidation.

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INFLUENCE OF STRESS-ULCER-PROPHYLAXIS ON TRANSLOCATION OF BACTERIA FROM THE INTESTINAL TRACT IN RATS. S.M. Feistauer, A.N. Laggner°, A. Makristathis, A. Georgopoulos
Univ. Vienna, Dept. of Chemotherapy and I.Dept. of Medicine, 1090 Vienna, Austria

Clinical studies in critically ill patients suggest an increased rate of septicemia during stress-ulcer-prophylaxis (SUP) with H_2-blockers, i.e. ranitidine, when compared with sucralfate, a topically active compound. Additionally increasing evidence exists, that translocation of bacteria (TLB) from the intestinal tract to mesenteric lymph nodes and systemic circulation occurs under several conditions, i.e. hemorrhagic shock. We wondered whether the mode of SUP affects TLB after hemorrhagic shock in rats.

In our study we used male Wistar rats (350g), who received standard meals without SUP (controls, n=8) and with sucralfate (400 mg/day, n=8) or ranitidine (10 mg/day, n=8) for seven days. At day eight hemorrhagic shock was induced and after 24 hours the animals were sacrificed to analyse bacterial flora in stomach, small intestine, colon, blood, mesenteric lymph nodes, liver and spleen. No significant differences were found in bacterial content of stomach, small intestine and colon between the three groups. However, in sucralfate treated animals , we found the lowest TLB rate ($p<0.05$). Therefore, sucralfate seems to prevent TLB after hemorrhagic shock in rats.

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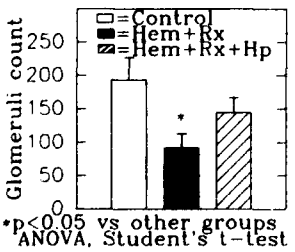
LOW MOLECULAR WEIGHT HEPARIN (LMWH) ADMINISTRATION DURING RESUSCITATION OF HEMORRHAGIC SHOCK IMPROVES RENAL MICROVASCULATURE. K.I. Chaudry*, G. Singh*, M.W. Rana*, I.H. Chaudry. Dept. of Surgery, Michigan State University, East Lansing, MI 48824.

Most models of hemorrhagic shock involve preheparinization. We have recently shown that preheparinization has protective effects on microvascular patency and on function of the liver, heart and kidneys after hemorrhage and resuscitation. Similar results were found even if heparin was given after hemorrhage, i.e. during resuscitation. These beneficial effects of heparin were not due to its anticoagulant properties. The present study investigated the effect of administering LMWH (2 mg/kg BW) to rats during resuscitation on renal microvascular patency to determine whether molecular weight of heparin is of any significance. The rats were divided into 3 groups: Group I (control) was sham-operated; Groups II (HEM+Rx) and III (HEM+Rx+Hp) were bled to and maintained at 40 mmHg by either withdrawal of more blood or infusion of Ringer's lactate (RL) until 40% of the shed blood volume had been returned in the form of RL. They were then resuscitated with 4X the shed blood volume with RL. Group III received LMWH at the onset of resuscitation. At the termination of the experiments, all rats were rapidly infused with colloidal carbon black. The kidneys were then prepared for histology. The results (Figure) demonstrate that the microvascular patency in the kidneys was significantly depressed in nonheparinized rats but was significantly improved in heparinized animals. Thus, the protective effects of heparin are not related to its molecular weight and the effects of even smaller, non-anticoagulant fragments of heparin require further investigation. (NIH GM 39519).



*$p<0.05$ vs other groups ANOVA, Student's t-test

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S4: Gut as a Source of MOF

57 TRANSLOCATION OF ENDOTOXIN AND BACTERIA FROM THE GUT FOLLOWING BURN INJURY. J. Wesley Alexander, M.D., Sc.D., University of Cincinnati Medical Center, Department of Surgery, Cincinnati, Ohio 45267-0558.

E. coli and E. coli endotoxin were labeled with ^{14}C glucose and used as translocation probes which were gavaged into the stomach immediately before a 30% burn injury was inflicted in mice. Animals were sacrificed at 1, 4 and 24 hours following injury. Translocation occurred extensively within one hour postburn and was greatest to the mesenteric lymph nodes followed by spleen, lung, and liver. Translocation of endotoxin followed a similar pattern except that less radioactivity could be found in the peritoneal cavity and more was found in the liver. Both E. coli and endotoxin translocated directly through the intact bowel wall to enter the peritoneal cavity. Killing of bacteria was greatest in the mesenteric lymph nodes and spleen. When Candida albicans was used to follow the translocation process in Thiry-Vella loops of burned guinea pigs and rats, the organism was found to pass through intact enterocytes rather than between them. The process of entry into the enterocyte was fundamentally different from phagocytosis and the process of extrusion into the lamina propria was fundamentally different from exocytosis. Free organisms from the lamina propria were frequently ingested by macrophages and could be found in both lymphatics and venules. It is concluded that microbes and endotoxin pass directly across intact enterocytes and that the estimation of translocation by viable bacterial counts in tissues grossly underestimates the extent of translocation of bacteria and ignores the translocation of endotoxin. Translocation of endotoxin may have a significant biological effect on the development of MOF which is independent of and in addition to the translocation of intact bacteria.

58 TRANSLOCATION OF INTESTINAL BACTERIA: MECHANISMS AND SIGNIFICANCE. C. L. Wells. University of Minnesota, Minneapolis, MN, USA, 55455

Bacterial translocation has recently been defined as the passage of viable and nonviable bacteria or bacterial products (such as endotoxin) from the intestinal tract to other somatic sites. Investigators have documented increased incidence of bacterial translocation following parenteral endotoxin; although intestinal histology is altered, the mechanism of endotoxin-induced bacterial translocation is not completely defined. Other experimental results have indicated that 1μ latex beads, *Escherichia coli, and Candida albicans* can translocate within tissue macrophages to the draining mesenteric lymph node; however, it is not yet known if macrophage transport is a primary or incidental mechanism of bacterial translocation. Translocating *Enterococcus faecalis* and *C. albicans* have been localized within intact ileal epithelial cells, indicating that the ileal enterocyte may be at least one portal of entry for translocating microbes. Using monoassociated mice, *E. coli*, *Proteus mirabilis*, and *E. faecalis* have been visualized within the tissue of the ileum and colon, suggesting that translocation may occur in both the small and large intestine. In these latter studies, viable *E. faecalis* was least efficient at translocating to the mesenteric lymph node compared to *E. coli* or *P. mirabilis*; in vitro studies with cultured intestinal epithelial cells indicated that *E. faecalis* adherence to the microvillous border was reduced, suggesting that epithelial attachment might be one factor influencing bacterial translocation. Current studies are designed to correlate results from animal models with results from in vitro studies (adherence, uptake, and intracellular survival) of translocating bacteria with macrophages and with cultured enterocytes.

60 **ASPECTS OF THE MECHANISMS OF BACTERIAL TRANSLOCATION IN A HYPOVOLEMIC-TRAUMATIC SHOCK MODEL IN BABOONS.** **G. Schlag, H. Redl, J. Davies*, H.P. Dinges and K. Radmore*.** Ludwig Boltzmann Institute Exp. Clin. Traumatol., Vienna, Austria; * Roodeplaat Reserach Lab., Pretoria, South Africa.
To study new aspects of the mechanism of bacterial translocation during traumatic shock we have investigated 10 male adult baboons (16 - 23 kg body weight).
Methods: The animals were anesthetised under EEG-control and instrumentated for hemodynamic measurements, for blood removal as well as for fluid administration. A flow probe (3 - 5 mm, Transonic) was placed into the superior mesenteric artery (SMA). The intramucosal pH of the gut (pHi) was measured with a Tonometer (Tonometrics Inc.) placed into the small intestine. Arterial blood samples were processed for microbiologic evaluation. Also liver, spleen and mesenteric lymph node samples were obtained aseptically at the postmortem examination for microbiological cultures. Hypovolemic-traumatic shock was produced by fractures, soft

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tissue trauma and hypovolemia for 3 hours followed by a retransfusion period of 3 hours.

Results:

	BL	Shock 1h	Shock 2h	Shock 3h	Reperfusion 1h	Reperfusion 3h
SMA-flow (med)	100	17.5	10.5	8.5	120.0	75.0
Gut-pHi (med)	7.35	6.95	6.65	6.55	7.07	7.15

Two types of responders could be observed: persistent low-flow and low pHi with corresponding gut damage (severe damage with low-flow) and high-flow (luxurious) during reperfusion with complete recovery of the pH.

Conclusion: The low-flow syndrome during the reperfusion period may be related to severe gut damage and to bacterial translocation.

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IMPORTANCE OF GUT ORGANISMS COMPARED TO OTHER FACTORS IN MULTI-ORGAN FAILURE. Benjamin F. Rush, Jr., University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Department of Surgery, Newark, New Jersey

Current evidence both clinically and from research models indicates that translocation of bacteria and/or endotoxin from the intestine plays a role in the induction of multi-organ failure (MOF). We have established a model of hemorrhagic shock in the germ-free rat which demonstrates that MOF occurs in the absence of bacteria but is more severe when bacteria are present. We have also shown that when hemorrhagic shock is maximal there is almost no effect from the presence of bacteria. However, when hemorrhagic shock is minimal or moderate the presence of bacteria markedly reduces survival and stimulates MOF. Other work from our laboratory indicates that both ischemia and sepsis stimulate the activation of complement and histologic changes in organs produced by ischemia or by ischemia plus bacteria.

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THE EFFECT OF HEMODILUTIONAL RESUSCITATION ON BACTERIAL TRANSLOCATION. L. Reed*, M. Martin*, M. Hochman*, F. Kaska*, R. Manglano*, J. Fildes, and J. Barrett. Cook County Hospital and University of Illinois, Chicago, IL 60612.

Hypertonic saline resuscitation(HSR) from hemorrhagic shock has been shown by our laboratory to reduce enteric bacterial translocation(TR). The aim of this study was to determine whether this inhibition is specific to hypertonic saline(HS), or the general effect of hemodilutional resuscitation. 180 Sprague-Dawley rats were anesthetized and subjected to either 30 or 90 min of hemorrhagic shock(30-50mmHg) via the Wiggers model. Resuscitation was performed with shed blood(B), lactated ringer's (3:1), 3%HS+ 1/2B(1:1), or 7.5%HS+1/2B(1:1). Spleen, liver, and mesenteric lymph nodes were sent for quantitative culture 24 hrs later. TR occurred if enteric organisms were cultured from at least 1 organ. Statistical analysis utilized the Fisher-Exact Test, with $p<0.05$ being statistically significant. p values were calculated comparing B with either HS or lactated ringer's(LR). Results are shown below.

RESUSCITATION	30 min +TR	30 min -TR	30 min %TR	30 min p	90 min +TR	90 min -TR	90 min %TR	90 min p
B	8	17	33		8	7	53	
LR	3	15	17	0.22	1	12	8	0.02
3%HS+1/2B	1	20	5	0.03	5	7	42	0.35
7.5%HS+1/2B	2	22	8	0.04	4	8	33	0.35

TR was inhibited by HSR after 30 min of shock, but not after 90 min. The opposite was true for LR. The incidence of TR with LR resuscitation after a 90 min shock period was significantly decreased. Hemodilution does, therefore, reduce TR. The beneficial effect of each resuscitative fluid, however, is dependent on the duration of hemorrhagic shock.

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THE EFFECT OF HEMORRHAGIC SHOCK ON INTESTINALLY DERIVED ENDOTOXEMIA. ANIMAL EXPERIMENTS. D. Nitsche*, C. Schulze*, C. Szeiki*
Dept. Gen. Surgery, Univ.-Hospital, D-2300 Kiel/Germany

Animal experiments were performed to clarify the role of the intestine as a source of endotoxins in hemorrhagic shock.

Methods: Anesthetized rats received E. Coli (2.5×10^{10} or 2.5×10^{11} cfu/ml/kg) through a duodenal tube and thereafter an antibiotic (Neomycin), also through the tube. A control group (A) received saline and no antibiotic. Immediately after the bacterial challenge, shock was induced by withdrawing blood until mean arterial pressure was reduced to 50 mm Hg. Plasma-endotoxin activity was measured every 30 min. for a total of 5 h after bacterial challenge.

Results: As early as 60 min. after administration of the antibiotic, an increase in plasma endotoxin activity (19,8 ± 9,6 EU/dl,n=12) was measured, in the sham shock rats (group B) that was significantly ($p = 0.03$)

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higher than in the saline control group(A)(6.1 ± 4.4 EU/dl,n=11). In this group, plasma endotoxin activity remained significantly raised until the end of the observation period. In the shock rats, increase in endotoxin activity one hour after shock was induced was significantly higher (43.8 ± 12.7; n=14) than in the sham-shock group. The increase in endotoxin activity in the shock groups was raised significantly (133.3 ± 24.5 n=9) when the dose of bacteria administered was increased to 2.5 x 10^{11}.
Conclusion: During shock endotoxins can enter the bloodstream from the intestinal tract. In such cases, antibiotic treatment increases the amount of endotoxins transferred.

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INTESTINAL DYSFUNCTION SEEMS TO ACCELERATE LIVER DYSFUNCTION IN HEMORRHAGIC SHOCK ?

F. Kobelt*, H.A. Henrich
Univ. Surg. Hospital., Dep. Exp. Surg., Josef Schneider Str. 2, 8700 Würzburg, Germany

In our Wiggers shock model in cats we investigated the intestine and a skeletal muscle two organs of different shock sensitivity in parallel. The arterio-venous (a-v) differences of intestinal blood parameters demonstrated that large amounts of ammonia (blood peak difference: 910 ± 237 µmol/l) and uric acid (blood peak difference: 2.3 ± 0.7 mg/dl)(measured by KODAK ECTACHEM DT60) are produced in this organ as compared to almost negligible quantities in the skeletal muscle. The kinetics of malondialdehyde (measured by the TBA method) exhibited an antiparallel behavior in both organs. The a-v-difference of Glucose was about 4fold in the intestine (peak value: 11.2 ± 1.3 mmol/l). Liver dysfunction, indicated by the redox potential (ß-OH-butyrate/acetoacetate) of liver mitochondria (MRP), started above a threshold of 4.9 ± 1.0 about 40 min and reached a maximum of 15.9 ± 5.3 about 90 min after onset of hemorrhage, just at the beginning of decompensated shock indicated by a significant blood reuptake.
Conclusion: In shock the intestine produces large amounts of toxic metabolites being released into the circulation and stressing the detoxification mechanisms of the liver in a period of low oxygen supply. As a results, liver dysfunction gradually increases up to a maximum at about 90 min after onset of shock.

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IS LIVER DYSFUNCTION RELATED TO DECOMPENSATION IN HEMORRHAGIC SHOCK ?

F. Kobelt, H. A. Henrich, Univ. Surg. Hospital., Dep. Exp. Surg., Josef Schneider Str. 2, 8700 Würzburg, Germany

In our Wiggers shock model in cats decompensation indicated by blood reuptake from the Lamson bottle started when the redox potential (ß-OH-butyrate/acetoacetate) of liver mitochondria (MRP) demonstrated a strong liver dysfunction. From this close relationship the question arose whether the liver might be involved in the initiation of decompensation. We investigated this question by applying a bolus of 10 mg/kg α-mercaptopropionyl-glycine (αMPG), a stabilizer of liver function, just after bleeding in the Lamson bottle had stopped (about 20 min after starting hemorrhage).
Results: In the shock group a significant blood reuptake of 0.95 ± 0.10 ml/min started at the maximum of the MRP value of 15.9 ± 5.3 about 90 min after hemorrhage. A comparable reuptake was found in the αMPG group about 150 min after hemorrhage.
The kinetics of other blood parameters like pH, glucose and triglycerides measured by standard methodes confirm this relationship.
Conclusion: The relation between liver dysfunction and the αMPG-sensible onset of decompensation points to a possible involvement of the liver in the changing physiology leading to decompensation in our hemorrhagic shock model.

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STUDIES ON INTESTINAL PERMEABILITY DURING HYPOPERFUSION IN PIGS.

E. Schlichting*, F. Næss*, M. Steinbakk*, T. Grotmol*, L. Bue*, T. Lyberg*
Ullevaal Hospital, 0407 Oslo 4, Norway.

Intestinal ischemia followed by reperfusion is an important and common clinical event. This intestinal injury appear to be central in the pathogenesis of multiple organ failure.
In a hemorragic 3 hour shock model or 5 hour clamping of the superior mesenteric artery model followed by reperfusion, we found just small amounts of endotoxin and no

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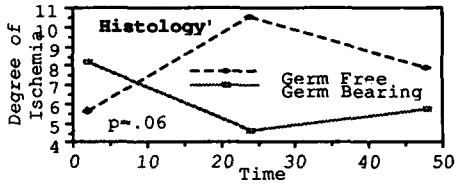
pathogenic bacterias in blood, lymph or lymphnodes. Regional hypoperfusion produced an extensive left ventricle failure. We further studied the permeability of intestinal mucosa after general or selective intestinal hypoperfusion. Segments of intestinal mucosa were mounted in Ussing chambers, and paracellular permeability was measured using probe molecules of differing molecular weights. Mucosae from ischemic intestines showed increased paracellular permeability in terms of augmented macromolecular flux and loss of electrical resistance compared to control animals.
Scanning electron microscopy showed marked alterations before any damage was evident at the light microscopic level.
Conclusion: Our experimental hypoperfusion models in pigs demonstrate morphological changes in the intestinal mucosa combined with increased permeability. Still, there are only very small amounts of endotoxin to be found in blood and lymph and no bacterial translocation taking place. This apparent discrepancy needs further clarification.

HISTOLOGIC ASPECTS OF HEMORRHAGIC SHOCK IN GERM FREE VS.CONVENTIONAL RATS. **67**

F.J.Ferraro*,B.F.Rush,J.McCullough*,T.Murphy*,J.Hsieh*,G.Machiedo,and K.Klein*.
University of Medicine and Dentistry of New Jersey, Newark, N.J. 07103

An increase in survival is found when germ free rats are compared to conventional rats following hemorrhagic shock. Histologic sections were studied to determine the differences by light microscopy. All rats were bled to a blood pressure of 30mm Hg. The germ free (n=14) and the conventional (n=9) rats were sacrificed two hours intrashock, 24 and 48 hours post shock. Slides were stained using hematoxylin and eosin. Applying a grading system based on severity of ischemic changes the kidney, spleen, liver,heart, small bowel and cecum were reviewed blindly by our pathologist. Using the sum of the changes of all organs the germ free rats showed greater ischemia at 24 hrs than the germ bearing rats. The kidney and lung showed the most severe changes. Germ free and germ bearing rats when compared histologically exhibit similar changes secondary to hemorrhagic shock, suggesting that the damage occurs secondary to a final pathway which may be stimulated by ischemia or bacterial *translocation. We suggest that the* greater ischemic changes seen in germ free rats is because of the significantly longer period in shock prior to decompensation ($p < .01$) and their significantly longer survival ($p < .01$).



THE EFFECTS OF GUT METABOLISM ON HYPERBIRIRUBINEMIA AFTER ESOPHAGEAL SURGERY. **68**

K. Setoguchi, T. Noguchi, S. Yoshitake, T. Kitano and N. Honda
Department of Anesthesiology, Medical College of Oita, Oita 879-56 Japan

This experiment was performed to determine the mechanism of postoperative hyperbilirubinemia (PHB) after esophageal resection with extensive lymphatic resection. The instestine plays a role as a barrier to enteric flora, preventing the host from invasion by bacteria and their toxins. During stress state, skeletal muscle releases glutamine and BCAA. Glutamin is a major fuel source used by the gut during critical illness and is necessary to maintain gut barrier function. In this study, plasma endotoxin and aminogram were measured during operation and postoperatively in 12 cases of esophageal cancer. PHB developed in 5 of 5 cases with positive plasma endotoxin. Glutamin and alanin were significantly lower in the patients with PHB than in the patients without PHB during operation. BCAA did not show any significant difference between the groups. These results suggested that lowering glutamin and alanin during surgical stress may be associated with derangement in the instestinal mucosa which is induced by a breakedown in gut barrier function. Hence, we concluded that the cause of PHB is translocation of endotoxin from the impaired gut mucosa.

EVIDENCE FOR PULMONARY ENDOTHELIAL CELL ATP DEPLETION FOLLOWING INTESTINAL ISCHEMIA-REPERFUSION **69**

Todd M. Gerkin*, Keith T. Oldham, and Karen S. Guice
Sections of General Surgery and Pediatric Surgery, Department of Surgery, University of Michigan Medical School, Ann Arbor, MI 48109

Notes

Intestinal ischemia-reperfusion is associated with clinical and experimental distant organ injury. Features of the pulmonary injury include in vivo parenchymal ATP depletion, neutrophil sequestration, and increased microvascular permeability. This study was designed to evaluate the hypothesis that a humoral factor(s) results in ATP depletion in the endothelial cell and that this injury may be reversible. Male Sprague-Dawley rats had intestinal ischemia induced by microvascular clip occlusion of the superior mesenteric artery (SMA) for 120 minutes. Reperfusion resulted from SMA clip removal. Following reperfusion for 0, 15, or 30 minutes, plasma samples were obtained from the portal vein. Time-matched sham operated animals served as controls. Monolayers of cultured rat pulmonary artery endothelial cells were incubated with 25% plasma. ATP levels were determined using a luciferin-luciferase assay. A ^{51}Cr-release assay using labelled cells was performed under identical conditions to determine cytotoxicity. Results:

GROUP	[ATP]	% CYTOTOXICITY
0/180	8.54 ± 0.68	1.6 ± 0.7
120/0	4.95 ± 0.76*	2.0 ± 0.5
120/15	10.13 ± 2.37	1.8 ± 0.4
120/30	9.80 ± 0.76	1.0 ± 0.3

(*$p < 0.05$ versus sham; [ATP] as moles/ug DNA X 10^{-12}; groups as minutes of ischemia/minutes of reperfusion) Endothelial cell ATP stores were depleted to 58% of control levels by plasma from the 120/0 ischemia-reperfusion group. However, despite this insult, no significant cytotoxic injury occurred. This data is consistent with the hypothesis that humoral factors, independent of the neutrophil, result in endothelial cell injury following intestinal ischemia-reperfusion. The injury is not lethal, however, and may be reversible.

70 ISCHEMIA/REPERFUSION INDUCED CHANGES IN THE SMALL INTESTINE; EFFECT OF LIMINAL PERFUSION ON THE SURVIVAL.

J. HAMAR, G. ILLYÉS, L. EGRI, K. KÁNTÁS, Z. DEMEL, and M. JUHÁSZ
National Institute of Traumatology, and Department of Surgery Central Hospital of the Police, Budapest, Hungary.

Occlusion/reperfusion of the superior mesenteric artery (SMA) is lethal in most of the cases. In our rat model one hour of ischemia was followed by the reperfusion of the SMA. We studied the sequence of events by semithin sections in the small instestine during the first 4 hours of reperfusion. In other series of experiments a slow luminal perfusion of the ileum and jejunum was initiated along with the release of SMA occlusion. Krebs (K) solution was used for control studies and 0.1, 0.2, and 0.4 mg/100 ml Lidocain was added to K in the treatment groups. Animals living more than 24 hours werre considered to be survivors. Histology of the gut showed desquamation of the villi during the first hour of reperfusion. At the same time epithelial regeneration started and it was completed in 3 hours. However, mucosal stroma showed an insreasing infiltration of leucocytes and a second desquamation of the epithelial layer was also observed. Luminal perfusion of the gut by K solution resulted in a increase of the survival rate form 0 % to 16 %. Increasing doses of Lidocain 0,1, 0,2, 0,4 mg in 100 ml of K increased survival by 20 %, 31 %, and 56 % in the respective treated groups. It is concluded that a two step damage of the mucosa takes place following ischemia/reperfusion that leads to mesenteric shock; survival can be increased by the removal of mediators and mediator release can be enhanced by the overactivation of the enteric nervous system.

71 MESENTERIC VASOCONSTRICTION IN RESPONSE TO CARDIOGENIC SHOCK - A PAN-MESENTERIC HEMODYNAMIC PATTERN (OVERVIEW OF A DECADE).

P Reilly*, RW Bailey*, GB Bulkley.
Johns Hopkins Medical Institutions. Baltimore, MD 21205

Cardiogenic shock is produced in pigs by controlled increases in pericardial pressure (tamponade). At maximal pericardial pressure, cardiac output ↓ to 42±2%, arterial pressure ↓ to 64±3%, & total peripheral resistance (TPR) ↑ to 136±6% of baseline (bl) levels. 39±5% of this ↑ in TPR is due to disproportionate pan-mesenteric vasoconstriction , manifest as an ↑ to 222±12% of bl in total mesenteric resistance, resulting in a ↓ in total mesenteric blood flow to 28±3% of bl (n=54). These changes are reflected quantitatively in the stomach, small and large intestine, and liver (both portal & hepatic arterial flows/resistances), and result in characteristic ischemia/reperfusion lesions in each (hemorrhagic erosions in the stomach and small intestine,ischemic colitis, and ischemic hepatitis) when shock is sustained 4h, followed by 2h resuscitation. Moreover, there is also injury to the lung (ARDS), myocardium, and kidney, an overall picture of multiple organ failure (MOF). Both this disproportionate pan-mesenteric vasoconstriction and the splanchnic and distant organ injuries are prevented by prior ablation of the renin-angiotensin axis (whether by nephrectomy, saralasin, or enalapril), but unaffected by ablation of either the sympathetic nervous system or of vasopressin. Therefore, the systemic hemodynamic response to cardiogenic shock is characterized primarily by disproportionate pan-mesenteric vasoconstriction that, while preserving flow to non-mesenteric organs, causes ischemic splanchnic organ injury which leads to the subsequent development of the multiple organ failure syndrome.

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ROLE OF BILE ACIDS IN THE PATHOGENESIS AND PREVENTION OF ENDOTOXIN SHOCK.

Lóránd BERTÓK
"Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiophygiene, Budapest, H-1775 P.O.Box 101, H u n g a r y

In healthy animals and humans the endotoxin can not absorb from the intestinal tract. However we have found that in bile deprived (common bile duct cannulated) rats the endotoxin (labeled with 3-H or 51-Cr) can absorb from the intestinal canal,and can produce a fatal endotoxin shock. The substitution of bile acids can prevent the absorption of endotoxin in bile deprived animals. Bile acid preatment can also prevent -in 70 per cent of dogs- the fatal intestinal ischemic (enteroendotoxemic) shock induced by superior mesenteric artery occlusion. The antiendotoxic effect of bile acids is based on their detergent activity. The regulation of bile excretion/flow by cholecystochinin and enterohepatic circulation play very important role in the homeostasis of macroorganisms. Disturbances of this regulation induced by various factors or effects (e.g. stressors, radiation, damage of small intestine, drugs etc.) could result the absorbtion of endotoxins from the intestinal tract. Prognosis of endotoxic shock depends from the quantity of absorbed endotoxins (their level in the blood). The detergent activity of bile acids is the basis of so-called physico-chemical defense mechanism of macroorganisms.

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THE NONINVASIVE DETECTION OF INTESTINAL ISCHEMIA WITH DUAL WAVELENGTH PHASE MODULATED SPECTROSCOPY.

J.B. Morris, H. Blum*, M. Maris*, E. Sevick*, B. Chance*.
Departments of Surgery, Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104

The substantial mortality of acute intestinal ischemia is due in large part to the failure of early recognition with irreversible tissue damage prior to therapeutic intervention. No screening diagnostic study is absolutely specific for the diagnosis. To this end, we employed the technique of dual wavelength phase modulated spectroscopy in a superior mesenteric artery (SMA) occlusion model of intestinal ischemia in the rat. The spectrophotometer emits modulated light (220 MHz) at 754 nm (peak absorption of deoxyhemoglobin) and 816 nm (near the isosbestic point where the absorption of oxyhemoglobin exceeds deoxyhemoglobin). Photons migrating through tissue are both scattered and absorbed producing alterations in mean photon pathlength detected as a phase shift. Anesthetized rats (N=8) were subjected to celiotomy, application of an SMA occluding device, and abdominal closure. Input/output fiberoptic probes were placed on the abdominal wall 1 cm apart. Following a pre-ischemic baseline total SMA occlusion was obtained. Results:

Wavelength (nm)	Pre-ischemic Voltage (mean ± SEM)	Ischemic Voltage (mean ± SEM)
754	-.0062 ± .003	-.0093 ± .003*
816	-.0008 ± .004	-.0006 ± .004

*p = .046 vs. pre-ischemic voltage at 754 nm (paired t-test)

The increased absorption at 754 nm by deoxyhemoglobin decreased mean photon pathlength producing a phase shift detected as increased negative voltage. This technique shows promise for the noninvasive detection of intestinal ischemia.

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MUCOSA AND SEROSA PO_2 OF THE JEJUNUM IN SWINE.

W. Hasibeder, M. Haisjackl, C.D. Schwarz, R. Häussler, S. Klaunzer, R. Germann, W. Lingnau, N. Mutz.
Univ. Innsbruck, Departments of Anesthesiology and Surgery, A-6020 Innsbruck

The intestinal mucosa is know to be extremely vulnerable to ischemia. One reason for this sensitivity is the counter-current arrangement of the microvessels in the villus leading to shunt diffusion of oxygen from the arterial to capillary and venous vessels. 12 pigs were anesthetized (sufentanyl, midazolam), paralyzed and normoventilated. Mucosa-pO_2 and serosa-pO_2 were recorded with a multiwire Clark-type electrode in an autoperfused segment of the jejunum. Arterial inflow was measured by an electromagnetic flow probe, the draining vein was cannulated. Intestinal oxygen transport (DO_2) and consumption were determined. PO_2 measurements were obtained if DO_2 was higher than the critical value. Critical DO_2 in this model has been determined to be 3.7 ml O_2/100g*min.

Results: Mucosa-pO_2 showed a rhythmic pattern with a frequency of 3-6/min which was in contrast to serosa-pO_2. Mucosa-pO_2 (mean 35.7, SD±8.9 mmHg) was lower than serosa-pO_2 (mean 65.0, SD±12.6 mmHg) at intestinal blood flows of 53.7 (SD±9.0) ml/100g*min and DO_2 of 5.7 (SD±1.2) ml O_2/100g*min.

Conclusions: Mucosa-pO_2 is much lower than serosa-pO_2 under non-ischemic conditions.The observed rhythmicity of mucosa-pO_2 may reflect blood flow changes due to villus vasomotion.

This study was supported by Leopold Inc., Austria.

Notes

S5: Relevance of Immunosuppression and Immunomodulation

75 ACTUAL ASPECTS OF TRAUMA-INDUCED IMMUNE ALTERATION AND COUNTERREGULATORY APPROACHES. F. Faist, M. Storck, A. Walz, D. Fuchs, H. Redl*, A. Markewitz, W. Ertel. Dept. of Surgery, LM University of Munich, Klinikum Großhadern and * Ludwig Boltzmann Institute Exp. Clin. Traumatol., Vienna, Austria

Monocytes (Mo) play a central part with the regulation of the immune response. In a study including 39 critically ill surgical patients (elective or emergency surgery, sepsis) we observed a deficit in IL-1 and IL-8 production several days after trauma (synthesis factor fIL-1 related to the percentage of Mo in PBMC cultures: 0.2 (D1), 0.3 (D3), 0.7 (D5); control value 3,6: $p<0.05$). We saw a considerable release of IL-6 which is a potent inducer of the acute phase reaction. There where maximum amounts of 19.6 x 103 U/ml IL-6 during sepsis (control 9.4±1.5 x 103 U/ml). Neopterin values were within the normal range in all cultures, suggesting intact cellular metabolism in circulating Mo. Serum Neopterin levels were significantly elevated during septic episodes. To obtain further insight into regulatory mechanisms of monokine and lymphokine release we correlated mRNA levels of some cytokines with the in-vitro protein synthesis.
In order to interrupt the posttraumatic inflammatory process, the administration of cyclooxygenase inhibitors has been proven to be effective. In a study including 60 patients undergoing open-heart surgery, we could reverse the deficit of in-vitro IL-2 production to normal values by combined therapy with TP-5 and Indomethacin. Administration of low-dose subcutaneous rhIL-2 with and without additional Indomethacin is also a therapeutic regimen currently investigated after major elective surgery.

76 A MECHANISM OF IMMUNOSUPPRESSION FOLLOWING TRAUMA LEADING TO IMPAIRED T-CELL ACTIVATION

D. Hoyt, A. Nuri Ozkan*, W.Junger*, W. Loomis*
Department of Surgery University of California, San Diego San Diego,CA 92103

Regulation of the inflammatory function, in general, by proteolytic fragments of matrix proteins following enzyme degradation is being increasingly recognized as an essential immunoregulatory mechanism.It is generally accepted that a number of low molecular weight biologically active factors are generated following traumatic injury. These factors have been demonstrated to elicit wide pathophysiologic abnormalities including suppression of the immune response. Injury leads to a wound based inflammatory process characterized by a provisional matrix rich in polymorphonuclear leukocytes and tissue destruction by neutrophils with accompanying proteolytic injury and the release of fragments with immunoregulatory activity. We have previously enriched peptides from patient's serum and from elastase digested fibronectin with a wide variety of activity. Further these have been shown to be released specifically after trauma and burn injury. In multiple previous studies, one well characterized peptide SAP has been shown to have direct suppressive influence on a variety of important cell types in the immune function.The mechanism of this immune suppression, however, is not completely clear. The detailed mechanisms behind the regulatory roll of SAP are not clear, however, our preliminary data suggests that the peptide interferes with the development of the cytosolic calcium signal.We have previously shown that the expression of activation antigens (IL-2R, TransferrinR and HLA-DR) is severely depressed following injury. The elevation of intracellular free calcium to a precise level is a prerequisite for induction of T cell activation antigen expression and blastogenesis. Current data suggests that inhibition of the mitogen induced increase in cytosolic Ca^{++} is due to decreased influx and decreased cytosolic release.

77 POSITIVE CORRELATION BETWEEN THE SEVERITY OF INJURY AND THE DEPRESSION IN CYTOKINE PRODUCTION FOLLOWING MAJOR MECHANICAL TRAUMA. W. Ertel, E. Faist*, C. Nestle*, M. Storck*, L. Hültner* [1], S. Alkan* [2], F.W. Schildberg* Department of Surgery and [1] GSF, University of Munich, Klinikum Großhadern, Munich, West Germany, [2] Department of Immunology, Ciba-Geigy, Basel.

Although it is known that, following major mechanical trauma, secretory function of T-lymphocytes is severely depressed, it is unknown whether there is any correlation between the extent of depression and the severity of injury. To determine this, 22 patients on days 3, 7, 10, 14 and 21 following mechanical trauma, were divided into 3 groups according to their injury severity score (ISS): group I<20 points (pts) (n=4) (mean 16.3±1.7 pts), group II 21-35 pts (n=9) (mean 29.8±1.3 pts), group III>35 pts (n=9) (mean 47.3±3.4 pts). Purified T-cell cultures were prepared. Lymphokine synthesis (Il-2, IFN-Y and Il-6) in T-cell supernatants was measured with bioassays. For normal values, 26 healthy controls were studied. Results are presented as cumulated data of all patients per experimental group: mean±S.E.M., *p<0.05 P vs C, # p<0.05 group II/III vs I, t-test:

	Control	Group I	Group II	Group III
Il-2 (U/ml)	1.07±0.12	0.42±0.11*	0.29±0.03*#	0.30±0.07*
IFN-Y (pg/ml)	5079±707	7439±865	2776±489*#	4011±629#
Il-6 (U/ml)	914±106	1018±167	2229±416*#	3135±509*#

One patient (group III; Iss:57 pts) died due to sepsis. In group II and III, Il-2 and IFN-Y release were significantly

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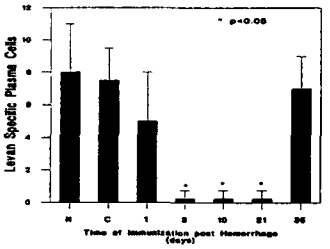
reduced compared to controls, while Il-6 release was significantly elevated. No significant differences between group II and III were found. IFN-Y and Il-6 release of group I were similar to controls, while Il-2 secretion was decreased to 50% of control values ($p<0.05$). These data clearly demonstrate that major trauma causes a severe depression of secretory T-cell functions in patients with major mechanical trauma (ISS>20 pts) independent on the ISS in group II and III. Patients with minor trauma (ISS<20 pts) only showed a suppression of Il-2 synthesis compared to healthy controls. These data indicate that the ISS correlates only partially with trauma induced immunosuppression in patients with mechanical trauma. Furthermore severity of injury alters lymphokine synthesis in a differential way.

HEMORRHAGE ALTERS BACTERIAL ANTIGEN SPECIFIC PULMONARY PLASMA CELL REPERTOIRES. **78**
E. Abraham, A.S. Robinson*. UCLA Medical Center, Los Angeles, CA 90024

Nosocomial pneumonia occurs frequently after hemorrhage and trauma and contributes to the increased incidence of morbidity and mortality after severe injury. The production of secretory antibodies by mucosally associated B cells is an important component of pulmonary host defense mechanisms. To determine the effects of hemorrhage on pulmonary B cell function, we examined hemorrhage induced alterations in bacterial antigen specific pulmonary plasma cell repertoires, using the bacterial polysaccharide levan (from Aerobacter levanicum). Levan specific plasma cells per 10^6 intraparenchymal pulmonary lymphocytes were determined by the ELISA Spot Assay. Hemorrhage of 30% blood volume completely suppressed the increase in levan-specific pulmonary plasma cell numbers normally present following oral immunization with antigen and adjuvant. These decreases in numbers of bacterial antigen specific pulmonary plasma cells were present between 3 and 21 days after blood loss (figure).



These results demonstrate that hemorrhage produces marked alterations in pulmonary B cell response, which may contribute to posthemorrhagic abnormalities in host defenses.

T-LYMPHOCYTE PROLIFERATION IS SUPPRESSED BY A CARDIODEPRESSANT FACTOR. **79**
W. Junger, S. Hallström*, F. Liu, W. Loomis, D. Hoyt and G. Schlag*.
*Univ. of California, San Diego, CA 92103. *Boltzmann Inst. f. Exp. and Clin. Traumatology, Vienna, Austria.*

Immunosuppression in trauma patients is considered to be a major cause of the development of sepsis. T-lymphocyte proliferation, a prerequisite of cellular immunity is markedly reduced in trauma and burn patients. Several groups have demonstrated that immunosuppression is related to circulating low molecular weight factors which are increased in shock plasma. A cardio-depressant factor (CDF) has been isolated from plasma of poly-traumatized dogs (see abstract by Hallström et al.). The purpose of this study was to evaluate effects of CDF on T-cell blastogenesis.
Peripheral blood mononuclear cells (PBMC) were isolated from blood of normal donors by density gradient centrifugation. Blastogenesis was determined in 96-well tissue culture plates: 10^5/well PBMC were incubated in 100 µl of supplemented RPMI-1640 medium with phytohemagglutinin as a T-cell mitogen (2 µg/well). CDF was tested as a 30-fold enriched concentration and at pathophysiologic level (dilution 1:32). After incubation for 72 h, ^{3}H-thymidine (1 µCi/well) was added; cells were harvested after 18 h; and ^{3}H-thymidine uptake was determined. 30-fold enriched CDF suppressed T-cell proliferation by 66.4±3.4% of the controls (mean±SEM, $p<0.002$). At pathophysiologic concentrations mean suppression was 17.1±7.5%.

	cpm	% suppression	
Control	16244±1533	0.0±9.4	
CDF trauma plasma conc.	13474±1218	17.1±7.5	
CDF 30-fold enriched	5461±558	66.4±3.4	(Values: mean±SEM)

Conclusion: These data suggest that CDF suppresses not only heart muscle performance but also may play an important role in the immune suppression on a cellular level.

DOES BLOOD TRANSFUSION (TX) OR HEMORRHAGIC SHOCK (HS) INDUCE IMMUNOSUPPRESSION? Jorge I. Cue,* James C. Peyton* and Mark A. Malangoni. **80**
Univ. of Louisville, Louisville, KY 40292

Although it has been suggested that blood transfusion may induce immunosuppression and increase the risk of infection, associated conditions (eg. sepsis, burns) in previous experimental models complicate this assessment. We studied the separate and combined effects of TX and HS on the immune response to infection in the absence of confounding factors. Lewis rats underwent either syngeneic or allogeneic (ACI donors) exchange TX of 10%, 20% or 30% blood volume with or without HS (MAP = 50mmHg x 45 minutes) and subsequent crystalloid resuscitation (n=15 for all groups). Immune response was assessed by: 1) abscess size 48 hours after intradermal injection of $2x10^7$ S. aureus; 2) migration of macrophages (M) into the peritoneal cavity following BHI injection; and 3) % Ia receptor expression of harvested M's determined by flow cytometric analysis. Data were

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compared using one-way ANOVA with followup t-tests. Syngeneic and allogeneic TX and various TX volumes gave similar results and were grouped for analysis.

parameter	control	TX	HS	TX + HS
abscess size (mm)	5.2±0.6	5.4±0.2	9.8±0.4*	9.7±0.3*
M ($x10^5$)	9.9±1.1	10.0±1.3	10.0±2.5	9.4±1.2
% Ia expression	15±2	88±5*	74±4*	83±14*

*p<0.05 v. control

These results show that HS increases abscess size while TX in the absence of HS has no effect on S. aureus abscess formation. Ia receptor expression on M's is stimulated by TX or HS, however, neither affects M migration. TX of 30% blood volume or less does not predispose to the development of staphylococcal bacterial infection in the absence of HS. Ia expression by M is stimulated by both TX and HS and does not correlate with infection risk or containment.

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IMPAIRED RES CLEARING FUNCTION AND CIRCULATING PROTEASE-ANTIPROTEASE COMPLEXES DURING TRAUMA. Å Lasson, K Ohlsson.
Depts. of Surgery and Surgical Pathophysiology, Malmö General Hospital, Sweden.

Various trauma result in the release of proteolytic enzymes, which are usually complexed to their appropriate inhibitors and thus instantly inactivated. The complexes are normally cleared by the reticuloendothelial system (RES). The aim of this study was to analyse possible circulating protease-antiprotease complexes in severe trauma and the rate of clearance of such complexes from the blood circulation in proteolytic diseases.

Material and methods: *Clinical study:* Plasma samples from patients during acute pancreatitis, after elective surgery and after urgent surgery because of peritonitis were analysed for possible protease-antiprotease complexes. *Experimental study:* Normal pigs got a slow iv. infusion of active trypsin, in amounts large enough to complex all their iv. alpha-2-macroglobulin, to stress the eliminating capacity of RES. Thereafter radio-labelled complexes of trypsin-alpha-2-macroglobulin were infused iv. The disappearance of radioactivity from plasma was measured.

Results: In the *clinical study* circulating complexes of protease-alpha-2-macroglobulin, kallikrein-alpha-2-macroglobulin, plasmin-alpha-2-macroglobulin, plasmin-alpha-2-antiplasmin, thrombin-antithrombin III and protease-C 1 inhibitor complexes were found during acute pancreatitis and in the postoperative period. Furthermore, low levels for alpha-2-macroglobulin and antithrombin III were found, indicating consumption of the protease inhibitors. In the *experimental study*, an impaired clearing of iv. infused radio-labelled trypsin-alpha-2-macroglobulin complexes was found after "preloading" of the RES, using slow iv. trypsin infusion prior to complex infusion. T 1/2 was prolonged from 5-7 min in healthy pigs to 100 min in pigs "preloaded" with iv. trypsin.

Conclusion: The eliminating capacity of the RES for protease-antiprotease complexes seems to be exhausted during trauma, resulting in large quantities of circulating protease-antiprotease complexes in plasma. Both the impaired RES function and the small, possible proteolytic activity of circulating protease-antiprotease complexes may have clinical significance during severe trauma.

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IMPAIRED NITRIC OXIDE PRODUCTION IN HEMORRHAGIC SHOCK: REVERSAL BY INTERFERON GAMMA. JB Ochoa, AO Udekwu, J Stadler, TR Billiar, RD Curran, T Kenkre, RL Simmons, AB Peitzman.
Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15261 USA

Macrophage-generated nitric oxide (·N=O) is essential for bacteriostasis. We have previously shown that septic patients produce large amounts of ·N=O but that patients in hemorrhagic shock (HS) do not. Furthermore, HS patients cannot produce ·N=O during subsequent septic episodes. Multiple other deficits in monocyte function follow injury and HS. The purpose of this study was to determine whether generation of ·N=O was impaired in macrophages after HS and whether this could be reversed by cytokine-mediated upregulation of macrophage function. Peritoneal macrophages (PM) were harvested from male Sprague-Dawley rats. ·N=O production was determined by assay of its stable metabolic product, nitrite (NO_2^-). NO_2^- production by PM harvested from control and HS rats in response to increasing doses of endotoxin was similar (data not shown). PM cultured in the presence of HS serum exhibited severely depressed NO_2^- production (Table 1). Addition of interferon gamma reversed this suppressive effect (Table 2).

TABLE 1

Time	Shock Serum NO_2^- (uM)	Control Serum NO_2^- (uM)	p =
Baseline	3.1 ± 1.1	2.6 ± 0.7	NS
24 hours	10.1 ± 1.5	13.6 ± 2.1	<0.01
48 hours	21.3 ± 1.5	32.4 ± 1.6	<0.01

TABLE 2

Interferon (u/ml)	Shock Serum NO_2^- (uM)	Control Serum NO_2^- (uM)	p =
0	20.0 ± 1.5	32.1 ± 1.1	.0001
10	35.2 ± 0.9	35.4 ± 0.9	NS
100	37.7 ± 0.9	39.1 ± 1.2	NS

Thus NO_2^- production by PM harvested from rats subjected to HS is normal. Furthermore, HS serum either lacks the interferon gamma necessary to maintain adequate macrophage function or contains a substance which inhibits NO_2^- production in PM.

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INTERACTION OF SOFT TISSUE TRAUMA AND UNSPECIFIC IMMUNESYSTEM IN POLYTRAUMATIZED PATIENTS. A. Seekamp*, G. Regel*, A. Dwenger*, G. Schweitzer*, J.A. Sturm*, (Spon: H.J. Oestern),
Unfallchirurgische Klinik, Klin. Biochemie, Medizinische Hochschule,D-3000 Hannover 61.

From clinical experience it is well known that patients with severe tissue trauma, be-

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sides multiple fractures, have a high risk of infection often followed by multiple organ failure. In 38 polytraumatized patients, mean ISS of 45.9, 21 (gr. B) of them with tissue damage of 2. or 3. degree according to the Hannover fracture scale (the others gr. A), we investigated polymorphnuclear granulozyte (PMNL) functions like cell count (CC) adherence (AD) opsonic capacity (OC) and chemiluminescence (CL) over a 14 day period every 12h. Attention was also given to Creatinkinase (CK) Lactatdehydrogenase (LDH) Elastase (Ela) alk. Phosphatase (Pho) c-reactive Prot. (CRP) and Neopterin (NEO). First of all CK and LDH had an initial peak of 1600 U/l respectively 600 U/l in both groups and therefore could not differentiate the severity of tissue trauma. PMNLs were overstimulated posttraumaticly in gr. B indicated by a significant decrease of CC until 30h, max. difference to gr. A 2.7x 10^6/ml. Also Zymosan induced CL response was significant deminished in gr. B the first 5 days while Ela normalized after an initial max. of 650 U/l parallel in both groups. While recovering from initial exhaustion, significant increase of CC from the 4. day on, AD in gr. B remained significant increased over the whole period whereas it normalized in gr. A from the 9. day. Also OC increased in gr. B from the 8. day on after initial similar values in both groups. In contrast Neo did increase almost parallel in both groups constantly up to 33mg/dl. In conclusion severe tissue trauma may cause a delayed sufficientphagocytosis, in gr. B significant increase of CRP from the 8. day to 35 mg/dl, followed by delayed fracture and wound healing, indicated by an increase of Pho only in gr. A from the 9. day on, resulting in a higher infection rate (gr. A: 52%, gr. B: 65%) and ending up in a significant higher lethality rate (gr. A: 23%, gr. B: 57%). Mean survival time was 9.3 days in both groups.

84 See page 168.

85 See page 168.

S6: Fluid Resuscitation

A COMPARISON OF ISOTONIC SALINE, HYPERTONIC SALINE, AND DEXTRAN-70 IN THE RESUSCITATION OF DOGS FROM ENDOTOXIC SHOCK. **86**

K.K. Nagy*, D.S. Kim*, J.M. Soyka*, M. Martin*, J. Fildes*, J. Barrett.
Cook County Hospital and University of Illinois College of Medicine, Chicago, IL 60612.

Hypodynamic septic shock was induced in beagles by the intravenous infusion of E. coli endotoxin (4 mg/kg). After 30 minutes of shock, the animals were resuscitated using 0.9% NaCl (NS, n=6), 3% NaCl (n=5), 7.5% NaCl (n=8) or 6% Dextran-70 (D70, n=5). There was no difference between fluids in hemodynamic parameters including mean arterial pressure, cardiac index or oxygen delivery after resuscitation. There was no difference in the hematocrit between groups. Although the pH in the hypertonic saline animals was markedly lower after resuscitation, this difference was not significant. The post-resuscitation serum sodium and serum osmolality was significantly higher in the two hypertonic saline groups when compared to NS and D70 ($p<.001$). In conclusion, there is no beneficial hemodynamic effect to support the use of hypertonic saline in endotoxic shock. In fact, the resultant hypernatremia and hyperosmolality may be deleterious to the septic animal.

HYPERTONIC SALINE-DEXTRAN AND THE RECOVERY OF HEPATIC BLOOD FLOW AND HIGH ENERGY PHOSPHATE CONTENT FOLLOWING HEMORRHAGE. **87**

W. Becker*, W. Cioffi*, A. Mason* and B. Pruitt.
US Army Inst. of Surg. Res., Ft. Sam Houston, TX 78234-5012

The clinical usefulness of small volume hypertonic saline-dextran (HSD) resuscitation following hemorrhage is unclear. Improvement in hemodynamic indices may be at the expense of cellular function due to shift of intracellular water. Following a 35% hemorrhage, the ability of small volume (4ml/kg) HSD to restore hemodynamic indices and hepatic adenosine triphosphate (ATP) content was compared to Ringers lactate (RL, 3ml/ml shed blood) and no resuscitation (NR) in immature swine. Resuscitation began 30 minutes following a 35% hemorrhage which decreased cardiac output (CO 2.28±.56 to 1.26±.4L/min. $p<.001$), oxygen delivery (O_2DEL 278±64 to 130±40ml/min. $p<.001$), hepatic blood flow (HBF 345±134 to 229±108ml/min. $p<.02$), hepatic ATP content (3.7±1.9 to 1.5±.4µmole/gm, $p<.001$) and mean blood pressure, (BP, 76±23 to 39±8mmHg, $p<.001$). One hour after resuscitation the following changes in hemodynamics were noted.

	NR (n=5)	RL (n=6)	HSD (n=5)
CO	1.2±.6	2.7±.83*	1.9±.5
O2DEL	134±55	247±80	184±69
HBF	189±103	388±112*	362±101
ATP	1.3±.3	2.5±.6*	3.5±1.2*
BP	42±13	60±13	58±5

*$p<.05$ compared to NR, mean ±SD

Conclusion: While HSD was less effective than RL in restoring CO and visceral blood flow, it was equally effective in restoring O_2DEL and hepatic ATP production. HSD may provide a brief period of organ support when standard resuscitation measures are impractical.

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THE EFFECT OF WATER DEPREVATION AND HEAT STRESS ON HYPERTONIC SALINE TREATMENT OF CONTROLLED HEMORRHAGIC SHOCK. MM Krausz, A Ravid, U Izhar, M Horowitz, D Gross, Hebrew University-Hadassah Med Sch. Jerusalem, Israel.

Hypertonic saline has been recently suggested for treatment of civilian as well as military trauma-induced hemorrhagic shock. The effect of hypertonic saline treatment of hemorrhagic shock following water deprevation and heat stress was studied in rats. The rats were divided into 4 groups: Gr. 1 (n=12) normal rats. Gr. 2 (n=15) water deprevation for 12 h (WD). Gr. 3 (n=13) heating at 37°C for 5 h (HS). Gr. 4 (n=14) WD and HS. Controlled hemorrhagic shock was then induced in all animals by arterial bleeding of 15ml/kg and the animals were treated after 15 min by either a) 5 ml/kg NaCl 0.9% (NS) or b) 5 ml/kg NaCl 7.5% (HTS). WD in gr. 2 was followed by fall in body weight (BW) of 5.3% ($p<0.001$) and rise in hematocrit (HC) to 49% ($p<0.01$). HS in gr. 3 resulted in rise of body temperature to 38.3°C ($p<0.001$) decrease in BW of 2.3% ($p<0.01$) and rise in HC to 52% ($p<0.001$). WD and HS in gr. 4 resulted in rise in body temperature to 39.8°C, fall in BW of 7.9% ($p<0.001$) and rise in HC to 54%. Arterial bleeding was followed by fall in mean arterial pressure (MAP) to 44±4 torr ($p<0.001$) in 5 min. Infusion of NS after 15 min in normal animals (Gr.1a) was followed by increase in MAP from 64±6 to 80±12 torr (not significant) while in Gr. 1b infusion of HTS was followed by increase in MAP from 57±7 to 86±5 ($p<0.05$). Infusion of HTS following WD (Gr.2b), HS (Gr.3b) or WD+HS (Gr.4b) did not lead to a significant rise in MAP. It is concluded that water deprevation and/or heat stress limit the hemodynamic response to HTS in controlled hemorrhagic shock.

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HYPERTONIC SALINE DEXTRAN (HSD) IMPROVES PERIPHERAL MICROVASCULAR PERFORMANCE FOLLOWING MASSIVE UNCONTROLLED HEMORRHAGE.

S.P. Bruttig*, D. Discher*, T.J. Doherty*, P. Borgström* and K.-E. Arfors*. Letterman Army Institute of Research, Presidio of San Francisco, CA and Pharmacia Experimental Medicine, La Jolla, CA, USA

Restoration of mean arterial pressure or cardiac output following massive hemorrhage generally requires twelve times the volume of Ringer's lactate (RL) or other isotonic fluids than of hypertonic/hyperoncotic saline dextran (HSD). Little is known, however, of the microvascular response to HSD or RL. Therefore, we studied systemic and skeletal muscle microvascular responses to massive hemorrhage in Hypnorm-sedated, urethane-anesthetized rabbits (0.8-1.2 kg). HSD or RL was administered following a 10 min control period and a 30 min spontaneous recovery from uncontrolled aortotomy hemorrhage. HSD increased mean arterial pressure, skeletal muscle blood flow and "intrinsic" microvascular reactivity. Modest to negligible increases in pressure, flow and reactivity were observed following administration of RL. Both HSD and RL hemodiluted the animals to a similar degree, despite an 8.5X disparity in administered volumes, and caused similar increases in respiratory rate. Therefore, these results appear to be independent of final vascular volume. Since previous studies have shown better cardiodynamic improvement with HSD, and since the present study indicates better post-hemorrhage perfusion of peripheral vascular beds with HSD rather than RL administration, a more effective total resuscitation from massive hemorrhage can be expected with HSD administration than with conventional fluid therapy.

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DEVELOPMENT OF EXTRAVASCULAR LUNG WATER (EVLW) AND O2-DELIVERY IN LAPAROTOMIZED SWINE.

M. Seyr, N. Mutz, B. Abendstein, W. Lingnau, C. Schwarz, C. Wieser, A. Dienstl
Univ. Innsbruck, Departments of Anesthesiology, Surgery and Internal Medicine, A-6020 Innsbruck

Introduction: Pulmonary problems following trauma or surgery are often claimed to be due to inadequate O2 delivery. This may result in early organ failure. It was the aim of the study, to search for correlates of DO2 and the development of EVLW subsequently to blood loss.

Methods: Systemic oxygen transport parameters and EVLW were studied in 12 juvenile swines which were undertaken enlarged bowel operations. Animals were anesthetized (sufentanyl, midazolam), paralyzed (pancuronium) and ventilated artificially during the whole experimental procedures. Hemodynamic parameters, measured via carotid line and Swan-Ganz catheter were kept constant by adequate infusion regimen of crystalloids throughout our experiments. EVLW was measured by means of a lung water computer (Am. Edw., Mod. 9305) using thermo-dye dilution technique. DO2 was calculated using Fick's formula.

Results: Despite constant macrohemodynamic parameters (pressures) , a decrease in DO2 due to blood loss (not substituted by blood) could be observed in 6 animals (group 1). This decrease in DO2 subsequently was followed by a significant increase of EVLW. In contrast to that, EVLW as well as DO2 remained stable in animals without any blood loss (group 2) as shown in table.

Table:	$DO_{2}0$	$DO_{2}end$	EVLW0	EVLWend
Group 1	15.5ml/kg	10.5ml/kg	6.4ml/kg	8.8ml/kg
Group 2	16ml/kg	16.8ml/kg	7.1ml/kg	6.3ml/kg

Conclusion: Our results show that a decrease in DO2 due to blood loss subsequently is followed by rising EVLW. This may indicate that early substitution of red cells may be necessary preventing initiation of lung disturbances leading to severe organ failure.

This study was supported by Laevosan Inc., Austria

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DOSE-RESPONSE COMPARISON BETWEEN HYPEROSMOTIC SALINE (HS) AND HYPERONCOTIC DEXTRAN-70 (HD) AS PLASMA VOLUME EXPANDERS. M.A. Dubick*, J.J. Summary* J.M. Davis*, J.Y.Greene*, C.E. Wade and G.C. Kramer.
Letterman Army Inst. Res., Presidio of San Francisco, CA 94129, and Univ. of Texas, Galveston, TX 77550, USA.

Despite the established efficacy of 7.5% NaCl/6% HD (HSD) in the treatment of hypovolemia, it has been suggested that the optimal formulation has yet to be realized. The present study investigates the cardiovascular (CV) effects of 0.9-25% NaCl and 3-24% HD, either alone or in specific combinations at a dose of 4ml/kg, in euvolemic sheep. Plasma samples were collected before, during and up to 60 min after infusion of the test solutions. Dose-response effects of HS were immediate increases in cardiac output (CO) of 30-85% and sustained increases of 10-35% over the 60 min, with no effect on mean arterial pressure (MAP). Plasma volume (PV) expansion with HS was an immediate, but transient increase of 12-35%. Infusion of HD induced sustained 10-20% increases in CO and 10-30% increases in PV, peaking 10 min post-infusion. HD also resulted in small (5-12 mmHg) increases in MAP. CV effects of HD correlated with a dose-response increase in plasma dextran concentrations. All HS solutions significantly increased plasma Na, which peaked a $\geq$200 mEq/l in the 25% group. The effects of both HD and HS together were additive on PV expansion and CO. These data indicate that higher concentrations of HS and HD than currently used have better PV expanding capabilities, but use of HS >7.5% may be limited by resulting hypernatremia and other effects.

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FLUID THERAPY FOR SUPERIOR MESENTERIC ARTERY OCCLUSION SHOCK IN RATS.

Y. Ohi, H. Bitoh and R. Ogawa.
Department of Anesthesiology, Nippon Medical School, Bunkyoku, Tokyo, Japan

The efficacy of three kinds of fluids such as isotonic sodium chloride (0.9%NaCl), hypertonic sodium chloride (7.5%NaCl) and iso-oncotic (3% in saline) alubumin solutions to maintain the hemodynamics was investigated in rats with superior mesenteric artery occlusion (SMAO) shock. Three fluids were infused so as to maintain the mean arterial pressure within 70% of control period. Animals in the control group without fluid therapy died within three hours because of shock. Three regimens could maintain the mean arterial blood pressure over three hours. The hypertonic crystalloid solution was effective in the early period of SMAO shock to regain the blood pressure by smaller volume. An excessively large amount of volume was required in the group infused with an isotonic crystalloid, providing inadequate tissue perfusion. The iso-oncotic colloid produced a stable circulatory status with minimum changes in tissue perfusion.

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HYPEROSMOLAR SOLUTIONS IN THE TREATMENT OF HEMORRHAGE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) L.Waagstein*, E.Wennberg*, H.Haljamäe, Department of Anesthesiology, Univ. Gothenburg, S-413 45 Gothenburg, Sweden.

We have previously reported that SHR have a reduced capacity to mobilize glucose following blood loss and that this disturbance is intimately correlated to early appearance of metabolic acidosis and short posthemorrhagic survival times. Hyperosmolar fluid treatment of shock in SHR should therefore be advantageous unless the high sodium load exerts deleterious effects on the hypertrophic heart of SHR. In the present study the efficacy in SHR of shock treatment with 2400 mOsm/L hypertonic saline (HS) and hypertonic glucose (HG) was therefore compared. Anesthetized SHR were bled 30% of their blood volume during a 20 min period followed by infusion of HS or HG (25% of shed blood volume) during a 10 min period. A control group of SHR were bled but untreated. The hemodynamic response was monitored and hematocrit, glucose, lactate, sodium, osmolality, acid-base values, as well as survival times, were determined. The efficacy of HS for shock treatment was superior to that of HG. The hemodilutional and hemodynamic effects of HS were more pronounced and its administration did not result in as enhanced lactate production as in the HG-treated group. The survival time of bled SHR treated with HS markedly exceeded that of HG-treated or non-treated animals. It is concluded that in spite of a deficient glucose mobilization of SHR in shock states treatment with HG is less efficient than treatment with HS. There may be differences in the distribution volumes and thereby in the plasma volume supporting effects between hyperosmolar sodium and glucose. A high glucose load in shock states may in addition enhance lactate production and cellular acidosis.

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HYPERTONIC SALINE (HTS)-INDUCED MECHANISMS LEADING TO INCREASED BLEEDING IN UNCONTROLLED HEMORRHAGIC SHOCK. Reuven Rabinovici*, Tien-Li Yue*, Michael M. Krausz, Teresa S. Sellers*, Karen Mackell Lynch*, and Gloria Feurestein. Dept. of Surgery Thomas Jefferson University Hospital,Phila. Pa 19107, SmithKline Beecham Lab, PA 19406, and Dept. of Surgery, Hadassah Hospital, Jerusalem.

HTS (7.5% NaCl) given to rats subjected to uncontrolled hemorrhagic shock (n=7) caused an initial favorable hemodynamic response followed by increased bleeding, shock and decreased survival. The increased blood loss resulted from: 1) increased vascular pressure and vasodilatation (total peripheral resistance index -27 ± 5% P<0.05) as initial bleeding occurred when MAP and cardiac index are increased vs. controls (+88 ± 10%, P<0.05, and +82 ± 7%, P<0.01, respectively), and as angiotensin II, a potent vasoconstricter, delayed the HTS-induced bleeding (resumed at 60 min); and 2) a defect in platelet aggregation reflected by decreased ADP-induced maximal aggregation (-79% vs. animals treated with 0.9 NaCl, P<0.05) and increased EC_{50} of ADP (+159%, P<0.05). These responses might be mediated at lease in part byProstacyclin as HTS-treated rats markedly elevated the 6-Keto-$PGF_{1\alpha}$/TXB_2 ratio (+140 ± 12%, P<0.01), and pretreatment with indomethacin decreased blood loss and improved MAP and survival. These data point out potential untoward hemodynamic and hematologic consequences of HTS treatment in traumatic injury with uncontrolled bleeding.

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OXYGEN CONSUMPTION IN UNCONTROLLED PRESSURE DRIVEN HEMORRHAGE IN DOGS. R Prist*, GA Braga*, IT Velasco, M Rocha e Silva. Research Division, The Heart Institute, São Paulo University, Caixa Postal 11450, São Paulo, Brasil.

Oxygen consumption, a major determinant in the outcome of hemorrhagic shock, was determined spirometrically in pentobarbital anesthetized dogs submitted to uncontrolled pressure driven hemorrhage (**INITIAL BLEEDING RATE**: 25 ml/min; thereafter, rate proportional to MAP). When blood loss reached 40 ml/kg (experimental zero time) dogs were randomized into 6 groups: **NT**: no treatment; **RL**: lactated Ringers infusion, 25 ml/min for 60 min; **HSD**: hypertonic NaCl (7.5%)-dextran 70, 6%, 6 ml/kg at zero time; **HSD-LR**: RL plus HSD; **HAD**: hypertonic sodium acetate (2400 mOsm/l = 10.7%)-dextran 70 (6%), 6 ml/kg in 4 min at zero time; **HAD-LR**: RL plus HAD. Table 1 shows that O_2 consumption is reduced to 61% of control by initial bleeding; NT dogs further decline to 25% of control. LR prevents further decline for 30 min; HSD and HSD-LR produce partial recovery, but only at 4 min; HAD and HAD-LR restore consumption to normal at 4 min, but in HAD-LR this recovery lasts for 60 min. Thus, HSD, added to the LR infusion slightly improves O_2 consumption, but HAD restores it to near normality.

EXPERIMENTAL TIME:		CONTROL	- 4 min	4 min	10 min	30 min	60 min
O_2 CONSUMPTION ml/(min.m^2)	NT	120±17	73±11	58±7	45±10	25±6	22±6
	RL	118±13	80±15	75±9	87±13	80±8	50±7
	HSD	108±11	56±5	88±14	68±10	40±4	34±6
	HAD	122±9	73±7	131±10	120±12	75±12	42±12
	HSD-RL	117±7	89±6	96±10	83±9	91±12	56±8
	HAD-RL	130±12	72±11	128±15	130±10	114±8	105±15

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HYPERTONIC SOLUTION IN HYPOVOLEMIC SHOCK: EFFECT OF EXPERIMENTAL PARAPLEGIA. RN Younes*, MM Itinoshe*, D Birolini* (Spon: M Rocha e Silva). Department of Surgery, University of São Paulo, São Paulo Brazil (LIM-62)

Hypertonic infusion was shown to be effective in reversing hypovolemic shock. A vagal afferent reflex originated in the lungs is important for the full hemodynamic response to hyperosmotic infusion. The present study evaluates the role of spinal cord pathways in conducting the efferent limb of the neural reflex following the infusion of hypertonic solutions for the treatment of hypovolemia. Male adult rats were submitted to complete interruption of the spinal cord at the level of C7. After hemodynamic recovery and stabilization (15 min), rats were submitted to hemorrhagic shock (mean arterial pressure (MAP) = 50 mmHg) for 30 min; they were then randomized into 3 groups: Control (CTL, n=10 - 4 ml/kg 0.9% NaCl infusion), Isotonic Solution (IS, n=10 - 32 ml/kg 0.9% NaCl infusion), Hypertonic Solution (HS, n=10 - 4 ml/kg 7.5% NaCl infusion). MAP (mm Hg, mean ± SE) was continuously monitored (Table I):

Group	Baseline	Spinal cod section	Preinfusion	minutes 10	30
CTL	138 ± 4	73 ± 2	50 ± 0	66 ± 3	55 ± 4
IS	135 ± 4	70 ± 5	50 ± 0	94 ± 6	64 ± 3
HS	139 ± 5	74 ± 4	50 ± 1	82 ± 5	61 ± 5

Infusion of HS or IS2 significantly increased MAP, compared with CTL, which remained unaltered. We conclude that the infusion of HS in hypovolemic rats increases MAP despite the interruption of efferent spinal tracts. The increase is transient (10-15min) and may be solely due to fluid shift and plasma expansion.

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EFFECT OF SPINAL CORD INTERRUPTION ON HEMODYNAMIC RESPONSE TO HYPERTONIC SOLUTION IN NORMOVOLEMIC STATE. RN Younes*, MM Itinoshe*, D Birolini* (Spon: M Rocha e Silva). Department of Surgery, University of São Paulo, São Paulo Brazil (LIM-62)

Apart from its effect on compartmental fluid shift, hypertonic infusion increases myocardial contractility and triggers a vagal afferent reflex which modulates the vascular response. This study evaluates the role of spinal cord pathways in conducting the efferent limb of the neural reflex following hypertonic solution infusion. Male adult rats were submitted to complete interruption of the spinal cord at the level of C7. After hemodynamic recovery and stabilization (15 min), rats were randomized into 4 groups (n = 10 in each group): Control (CTL, no treatment), Isotonic Solution 1 (IS1, 4 ml/kg 0.9% NaCl infusion), Isotonic Solution 2 (IS2, 32 ml/kg 0.9% NaCl infusion), Hypertonic Solution (HS, 4 ml/kg 7.5% NaCl infusion). Mean arterial pressure (MAP: mm Hg, mean ± SE) was continuously monitored (Table I):

Group	Baseline	Spinal cord section	Preinfusion	minutes 10	30
CTL	122 ± 3	83 ± 8	96 ± 8	95 ± 8	89 ± 6
IS1	126 ± 5	74 ± 7	94 ± 7	93 ± 7	83 ± 7
IS2	128 ± 4	88 ± 6	99 ± 4	110 ± 6	85 ± 4
HS	127 ± 3	63 ± 8	85 ± 5	108 ± 7	89 ± 4

Similar transient increases in MAP occurred after HS or IS2 infusions. CTL and IS1 caused no significant alteration in MAP. We conclude that the infusion of HS increases MAP despite the interruption of efferent spinal tracts, probably due to fluid shift and plasma expansion.

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PRE-HEMORRHAGE INFUSION OF HYPERTONIC/HYPERONCOTIC SOLUTION DELAYS SEVERE HYPOTENSION IN DOGS. RN Younes*, VM Yoshida*, E Minami*, F Aun*, D Birolini* (Spon: M Rocha e Silva). Department of Surgery, University of São Paulo, São Paulo Brazil (LIM-62)

Prehospital treatment of hypovolemic patients with hypertonic NaCl-Dextran (HSD) solutions immediately improves the hemodynamic parameters of shock. The rapid plasma expansion following the administration of these solutions suggests that the infusion of HSD might delay hypotension if infused to a normovolemic animal prior to hemorrhage. The present study evaluates the effect of hypertonic infusions, given to normovolemic dogs, on the total volume of shed blood required to reduce mean arterial pressure (MAP) to 40 mm Hg. Thirty pentobarbital anesthetized mongrel dogs were monitored for mean arterial pressure (MAP) and randomized into 3 groups: CTL (Control, n = 10, infusion: 4 ml/kg 0.9% NaCl); HS (Hypertonic Saline, n = 10, infusion: 4 ml/kg 7.5% NaCl); and HSD (Hypertonic Saline + Dextran, n = 10, infusion: 4 ml/kg 7.5% NaCl + 6% Dextran 70). Following infusion, dogs were bled from the femoral vein until MAP reached 40 mm Hg. The dogs were maintained at MAP = 40 mm Hg during 30 minutes. The total volume of shed blood was measured. HSD treated dogs required a significantly greater volume of bleeding to reach shock level (65.2 ± 3.7), compared with HS (45.1 ± 4.8) and CTL (51.4 ± 3.8) groups (ANOVA, $p < 0.01$). These results suggest that the infusion of HSD into "potentially" hypovolemic subject might prevent, or delay severe hypotension, conceivably by expanding intravascular volume and affecting other hemodynamic and cardiovascular parameters.

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UNCONTROLLED HEMORRHAGE PRODUCED BY A TAIL CUT IN RATS: EFFECTS OF DIFFERENT ANESTHETICS. MCV Bilynskyj*, ML Errington*#, IT Velasco, M Rocha e Silva. Research Division, Instituto do Coração, Caixa Postal 11450, 05499, São Paulo, Brasil and Division of Neurophysiology, National Institute Medical Research, London, NW7, UK(#).

Krauzs et al claim that 7.5% NaCl (HS) resuscitation is detrimental to neurolidol-ketamine (NK) anesthetized rats undergoing uncontrolled hemorrhage produced by cutting of the tail at 12% (T12%) or 50% (T50%) from its tip. The effect of such hemorrhage was re-examined in experiments performed on male Wistar rats (235-345g), anesthetized with NK (1.5mg/kg/12mg/kg), pentobarbital (50mg/kg P), chloralose (40mg/kg C), or urethane (1.5g/kg U), n = 20 in each anesthetic group, half of them treated with HS (4 ml/kg IV) 15 min after start of bleeding. Mean arterial pressure (MAP) and cumulative blood loss were monitored for 195 min after the tail cut. NK induced large cumulative blood loss (34 ml/kg in T50%, and 17 ml/kg in T12%) and hypotension (70 mmHg drop in T50%; 25 mmHg in T12%). Mortality was 3/10 (T50%) and 2/10 (T12%). HS produced a significant transient recovery of MAP and increased blood loss (to 39 and 22 ml/kg for T50% and T12% respectively), but mortality was not significantly different from controls (5/10 in T50%; 3/10 in T12%). The other 3 anesthetics produced only mild cumulative blood loss and slight hypotension, which was not affected by HS. Only 3/60 rats died under these anesthetics (2 HS treated, 1 untreated). In a supplementary pentobarbital anesthetized group, blood was forcibly removed from a large artery to mimic, throughout, the blood loss observed in the NK-T50% group. Hypotension and death rates were comparable (4/10 HS treated, 1/10 untreated). It is concluded that the effects of NK are mostly due to its powerful vasodilator effect, which is apparently sufficient to impede the normal vasoconstrictor response to shock.

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Notes

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STUDY OF EFFECT OF THE NEWLY DEVELOPED ARTIFICIAL BLOOD "NEO RED CELLS (NRC)" ON HEMODYNAMICS AND BLOOD GAS TRANSPORT IN CANINE HEMORRHAGIC SHOCK. A. Usuba, R. Motoki, K. Suzuki*, Y. Miyauchi* and A. Takahashi*.
First Dept. of Surg, Fukushima Med. College, 1 Hikarigaoka Fukushima-city 960-12 Japan *Terumo Corp., 1500 Inokuchi Nakai-machi, Ashigarakami-gun, Kanagawa 250-01 Japan

Neo Red Cells (NRC) is the so called artificial blood for which no inhalation of 100% oxygen and no judgement of blood type or cross match test is required and whose storage for more than 1 year is possible. The purpose of this study is to evaluate the newly developed liposome-encapsulated hemoglobin, named Neo Red Cells (NRC), in the treatment of hemorrhagic shock. The particle size of NRC is 180±80nm, the hemoglobin concentration is 5.6mg/dl, the viscosity is 2cp and P_{50} is 49.5mmHg. The experiment was carried out on six mongrel dogs suffering hemorrhagic shock. Blood was extracted from the femoral artery and the animals lapsed into shock state. When blood pressure became lower than 60mmHg, NRC in amount equal to the amount of blood extracted was transfused immediately. Inhalating normal room air, the above manuplation was repeated 3-5 times. After 59% to 88% blood exchange using NRC, the total peripheral vascular resistance index (TPRI) was reduced and the cardiac index (CI) was increased, thereby alleviating the burden on the heart. The reduction of TPRI in the presence of hemorrhagic shock is presumed to be due to the small size of the NRC granules and their low viscosity. As the exchange rate increased, the oxygen consumption (VO_2) increased remarkably, presumably due to the increase of CI and the A-V difference of oxygen content. When the exchange rate was 88% the NRC-hemoglobin VO% was 60.4%. The conclusion of the study is that NRC is more suitable than natural blood for the treatment of hemorrhagic shock.

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PENTASTARCH VS. RINGER'S LACTATE IN THE RESUSCITATION FROM HEMORRHAGIC SHOCK IN HUMANS. K.K. Nagy*, J. Davis*, M. Martin*, J. Barrett.
Cook County Hospital and University of Illinois College of Medicine, Chicago, IL 60612

Pentastarch is a colloid which is chemically similar to Hetastarch. It has a shorter half-life (12 hours) and produces volume expansion at least 1.5 times the administered volume. We compared Pentastarch to Ringer's Lactate in 41 patients presenting with hemorrhagic shock. The groups were similar in age, sex, race and type of injury. Significantly less volume of Pentastarch was required initially to resuscitate to a normal blood pressure and urine output than Ringer's Lactate ($p<.005$). Coagulation parameters (protime, partial thromboplastin time, fibrinogen and factor VIII) were measured for 48 hours post-resuscitation and no abnormalities were noted in the Pentastarch group. Serum albumin was the same in both groups throughout the study period, however serum colloid oncotic pressure was elevated at one hour post-resuscitation in the Pentastarch group ($p<.005$). There was no difference in ventilatory parameters, blood gases, pulmonary function tests, ventilator days or hospital days between the two groups.

	Volume (cc.)	Hospital	Ventilator	PT (12 hr.)	PTT (12 hr.)	COP(1°)
Pentastarch	1750 *	10.6 d.	32.5 hr.	13.5	36.0	24.6 *
Ringer's	3629	9.1 d.	33.1 hr.	13.6	39.6	13.6

(* = $p<.005$, ANOVA)

We conclude that Pentastarch is safe and effective for the initial resuscitation from hemorrhagic shock.

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HYPERTONIC SALINE VERSUS LACTATED RINGER'S SOLUTION RESUSCITATION AFTER HEMORRHAGIC SHOCK IN RATS: EFFECTS ON THE FLASH EVOKED POTENTIAL. M. Matteucci*,D. Wisner and D. Woolley*.
Univ. California at Davis, Davis, CA 95616

In resuscitation from hemorrhagic shock, the use of very small volumes of hypertonic saline(HS) improves blood pressure and reduces edema: Effects on brain function as measured electrophysiologically, however, have not been studied. This study examined the effects of hemorrhagic shock and resuscitation with HS or lactated Ringer's(LR) solution on the flash evoked potential(FEP). Rats were hemorrhaged to a mean arterial pressure (MAP) of 37 mm Hg for one hour, then resuscitated with HS(n=10) or LR(n=10) to a MAP of 80 mm Hg for another hour. Latencies in both groups increased during shock and returned towards baseline during resuscitation. Amplitudes decreased during shock and increased towards baseline during resuscitation. There were no consisent differences between groups, although HS tended to restore the FEP better than LR. To resuscitate the rats to a MAP of 80 mm Hg, 3.8±0.5 ml/kg HS and 42.9±7.5 ml/kg LR ($p<0.05$) were required. Neural tissue dehydration during HS resuscitation had no apparent deleterious effects on acute brain function as measured by the FEP.

Notes

S7: Scoring Systems

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COMPARISON OF APACHE II, SEPSIS SEVERITY SCORE AND MOF-SCORE (GORIS) IN POSTTRAUMATIC MULTIPLE ORGAN FAILURE.

M.L. Nerlich, M. Holch, M. Maghsudi, Department of Trauma Surgery, Hannover Medical School, Hannover, Germany

Onset and quantification of multiple organ failure (MOF) is not defined clearly in trauma patients. Prognostic value, prospective applicability and practicability of use were compared in three different scores, currently available for use in the intensive care unit. APACHE II (A), Sepsis Severity Score (S) and MOF-score (M) were calculated daily (day 1-14) in 35 multiple trauma patients (mean ISS= 29.3). Two groups were established according to an analysis of discriminance of 25 variables describing a septic state: 18 septic and 17 non septic patients (lethality 72%, resp. 5%). All three scores used showed significant differences between both groups. Using the scores, a trauma related phase of organ insufficiency can be separated from a secondary insult going along with the septic reaction starting at the third to fourth day after trauma. The extent of failure of separate organes can be demonstrated with the (M). It shows a sensitivity of 62.6% and a specificity of 76.3% (cut off: 4.0 points). (A) revealed significant differences between the two groups at first before clinical onset of septic state. Sensitivity and specificity of (S) was lower than in (M) and (A). The practicability of (M) was superior to that of (A) or (S) as (M) was the only score that allowed a differentation into organ-specific subgroups.

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CLASSIFICATION OF THE SEVERITY OF INJURY: THE "POLYTRAUMASCHLÜSSEL" PTS

H.-J. Oestern und K. Kabus
Unfallchirurgische Abteilung, Allgemeines Krankenhaus, 3100 Celle

The PTS was developed in 1983 as a predominantly anatomical score for trauma victims. More than 700.000 data collected from 696 multiple-injured patients were evaluated by discriminant analysis. The result was a one-page chart rating the most frequent injuries and the age according to their importance to the outcome.
The PTS was critically reviewed in 1989. As a result, the rating of some injuries, especially of the extremity fractures, was found to be no longer up-to-date and had to be lowered. Furthermore, the accuracy of prediction has been increased by integrating the base excess and the quotient of p_aO_2 and F_iO_2 at admission.
The revised PTS, the Injury Severity Score, the Revised Trauma Score and the TRISS-method were then compared in a prospective study containing 155 seriously injured patients. While there were no significant differences in specifity among the scores, the revised PTS had the highest sensitivity, predicting correctly 80 per cent of the non-survivors.
A compact anatomical score including the patients' age is easily and early to apply and allows correct prediction of the outcome in a rate similar or superior to methodically more sophisticated classifications. The integration of objective parameters of vital functions further improves the accuracy of prediction by considering individual reaction on trauma, free interval and quality of prehospital care.

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PREDICTION MODEL OF POSTTRAUMATIC LUNG FAILURE BY C3A AND ALPHA-2-MG LEVELS IN BRONCHOALVEOLAR LAVAGE FLUIDS

Th.Joka,U.Obertacke,E.Kreuzfelder[1],M.Kirschfink[2] and F. Rumler
Dep.of Traumatology Univ. Essen,Dep. Immunology, [1]Univ.Essen,[2]Univ.Heidelberg FR Germany

The mechanisms of posttraumatic lung failure were investigated by a prospective clinical study including 25 trauma patients with an injury severity score over 40 points. Cellular and humoral parameters, such as plasma proteins (i.e. alpha-2-macroglobulin) and inflammation mediators (i.e. complement split product C3a) have been observed by daily bronchoalveolar lavage (BAL) starting 6 hours after trauma. Progressive posttraumatic lung failure (ARDS) was defined according to the "clinical score" given by MURRAY (ARRD138(1988) 720).

RESULTS: Combining the clinical score of lung failure and local concentrations of biochemical parameters (C3a and alpha-2-MG) in BAL-fluid, a prediction model of ARDS for a single patients, within the first 24 hours after trauma was constructed: The first precondition was a clinical score of lung failure higher than 2,5; ("early impairment of lung function").

The second precondition is the increasement of borderline alveolar concentration by alpha-

Notes

2-MG > 0,15 mg/ml ELF and/or by complement split product C3a > 6µg/ml ELF ("alveolocapillary protein leakage and/or local alveolar inflammatory reaction").
According to this model, 12 out of 14 prospectively tested patients could be correctly predicted to later ARDS or non-ARDS within 24 hours after trauma. All 8 patients with later ARDS could be predicted within this time,2 patients were falsely positive predicted by not developing an ARDS. The prediction model ist validated in a further prospective study.

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OUTCOME PREDICTION IN ICU TRAUMA PATIENTS. M.J. Vassar*, C.A. Perry*, F.R. Lewis, A. Clinton* and J.W. Holcroft.
Depts. of Surgery, Univ. of Calif., Davis, 95817 and Univ. of Calif., San Francisco, 94143.

The APACHE II (AP II) system has been proposed as a tool for predicting outcome in ICU patients, including trauma patients. We prospectively compared the AP II system with a proposed outcome index based upon points assigned for 3 variables collected at 24 hours after ICU admission: PaO_2/FiO_2 index, Glasgow coma score, and fluid balance. Using logistic regression the predictive power of AP II, and the proposed 24-HOUR ICU point system were compared using a decision criterion of > 50% for risk of death.

	Predicted To die	Predicted To live	Sensitivity	Specificity	Classified Correctly*
APACHE II					
Actual deaths	62	75	45%	99%	44%**
Actual survivors	8	855			
24-HOUR ICU POINTS					
Actual deaths	86	51	63%	98%	61%
Actual survivors	19	844			

* Computed from the Youden Index; ** $p < 0.005$

Conclusion: This study has included over 600 more trauma patients than the original AP II studies. The AP II system significantly underestimated the mortality in the 137 non-survivors. Efforts to develop scoring systems that more accurately represent the ICU trauma patient population are warranted.

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IS THERE A PATTERN OF ORGAN FAILURE FOLLOWING SEVERE TRAUMA ? - A POST MORTEM EXAMINATION.H.-C.Pape, G.Regel, J.A Sturm H.Tscherne (Sp:H.J. Oestern) Dept of Traumatology Hannover Med.School, FRG INTRODUCTION: Multiple organ failure (MOF) is characterized by sequential loss of organ function resistant to therapy. Descriptions of the sequence of organs involved are variable. We therefore evaluated clinical parameters of organ function, post mortem organ weights and histological specimens of organs from deceased trauma patients.MATERIAL AND METHODS: 81 patients, deceased after severe trauma, were distributed: I: death<24 hrs; II: death<96 hrs; III: death>4 days. Parameters: Lung, Horovitz ratio (Pa_{O2}/FiO_2) Liver, S-Bilirubin (umol/l) Kidney, S-Creatinin (umol/l) Heart, Cardiac index (ml/min/m²). Clinical data 1 day before death are shown. Organs were weighed post mortem (wt., /gramm) and histology was obtained (N= cell necrosis in % of total specimens).
RESULTS:

	Lung	Liver	Kidney	Heart	Pneum.	Sepsis
I	372,02	22.4	120	10.25	5.3%	0%
II	43,14	25.5	110	9.40	12.5%	38%
III	95,60	125.0	179	8.53	16.95%	44%

	lung			kidney			liver			heart		
	I	II	III	I	II	III	I	II	III	I	II	III
wt/g	1253	2284	2647	362	427	398	1653	2164	3001	330	440	395
N/%	21.1	25	25	21.1	51	59.4	31.6	37.5	65.6	42.8	87.5	68.8

CONCLUSIONS: 1. The lung was the earliest organ failing after severe trauma, in the late phase liver and heart failure occurred. There was no pertinent time of kidney failure. 2. A good correlation of organ weight and cell necrosis was seen in all organs. 3. Clinical patterns of organ failure did correlate with morhologic parameters in kidney, liver and heart, but not in the lung - here different pathomechanisms may be important.-

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MULTIPLE ORGAN FAILURE, THE CONSEQUENCE OF A WHOLE BODY INFLAMMATION AFTER MULTIPLE TRAUMA. G. Regel, H.C. Pape, U. Lehmann, F. Koopmann, J.A. Sturm (Spon: H.J. Oestern). Department of Trauma Surgery, Medizinische Hochschule Hannover, Konstanty Gutschowstr.8
Multiple organ failure (MOF) is a cumulative sequence of organ failures frequently seen in association with shock after multiple trauma. Similar to the known mechanisms for lung injury (ARDS), the activation of polymorphonuclear leucocytes (PMNL) and the release of toxic oxygen radicals at the vascular endothelium are also supposed to lead to a generalized loss of cell integrity and organ function in MOF.
Method: To prove this hypothesis and the possible timepoint of injury, we looked at the autopsy specimens of multiple trauma patients. 59 patients, dying immediately (Gr. I), 24 to 96 h (Gr. II) and >96 h (Gr. III) after multiple trauma were investigated. Next to organ weight, histologic sections were analysed with special attention to PMNL and monocyte accumulation, permeability edema and tissue damage (necrosis).

Notes

Results: % of total specimens	Lung			Kidney			Liver			Heart		
	Gr. I	II	III	I	II	III	I	II	III	I	II	III
PMN	57.9	62.5	50	4.7	6.8	6.3	5.3	12.5	18.8	2.4	5.6	6.8
Edema	57.9	87.5	43.8	5.3	12.5	3.1	5.3	18.3	10.7	42.1	25.0	46.7
Necrosis	21.1	25	25	21.1	50.0	59.4	31.6	37.5	65.6	42.8	87.5	68.8
Org.Wght.(g)	1253	2284	2647	362	427	398	1653	2164	3001	330	440	395

Discussion: An accumulation of PMN's was found in all organs immediately after trauma. Parallel to this an increase of permeability edema was seen with a maximum at the 4. day after trauma (Gr. II). In this group edema leads to a fulminant onset of organ failure and to early death. Tissue damage (necrosis) is seen with a maximum: initially (lung), or further on: 2.-4. day (heart) and >4 days (liver, kidney) in correspondence to the clinical onset of organ failure. Group III shows the prolonged development of MOF with a maximum of necrosis formation and organ weight.

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DAILY APACHE II (AP) SCORING (Scg) IN SEPSIS (Se) AND SEPTIC SHOCK (SeSh): VALIDATION AS CRITERION FOR INTENTION TO TREAT AND FOR RESPONSE (Re) TO THERAPY (Th). K. Werdan and G. Pilz. Dept. of Medicine I, Grosshadern Univ. Hospital, Univ. of Munich, Germany

Beside the use of initial AP Scg as a prognostic tool, daily score (Sc) measurements should provide an approach to identify risk patients (P) and to quantify the Re to Th.

AP Sc as criterion to identify risk P with intention to treat in cardiac surgery (CS): In 110 non-selected P after CS, daily AP Scg was studied with regard to its usefulness in the early diagnosis of P at risk (n=16), mainly due to Se (Elebute Se Sc ≥12 on ≥2 days), with a significantly worse prognosis (mortality (M) 69% vs 1%). Compared with single parameters (e.g. fever, leucocytes, neopterine and PMN-elastase plasma levels), AP Sc measured 24h post surgery (day 1) was superior in separating P at risk and P with uneventful course, resp. In a consecutive study carried out prospectively (n=106 P), an AP Sc of ≥19 on day 1 could be confirmed as a discriminating criterion (M 36% vs 0%).

AP Sc as criterion for Re to Th: The effects of supplemental immunoglobulin Th in P with Se and SeSh on disease severity (multiple organ failure) were investigated by means of daily AP Sc measurements in a multicenter study with a total of 131 P. In about half of the P (responders (R)), a prompt improvement (mean AP Sc decrease by 8.0) was evident from day 0 (onset of Th) to day 4, being associated with a better prognosis (M: R 24%, non-R 58%). A fall in AP Sc of ≥4 on day 4 was suited to classify R to Th.

Practicability of AP Sc: Despite their usefulness for quantitative evaluation, the use of Scg systems such as AP Sc has not become daily ICU routine, the lack of practicability being a main reason. A pocket-computer based program written in BASIC enables the ICU physician, even if unexperienced in Scg, to calculate AP Sc (as well as Elebute Se Sc) within 5-10 minutes in a bedside manner, including data outprint and storage.

Conclusions: In Se and SeSh, daily AP Scg is a practical, promptly available tool to identify risk P with the intention to treat as well as to classify the response to Th.

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TRAUMA SCORING - A PROSPECTIVE TRIAL FOR VALIDATION. B.Bouillon, A.Lechleuthner, M.Schweins, M.Vorweg (Spon. E. Neugebauer)
Univ.Cologne, II[nd].Department of Surgery, D-5000 Köln 91

The concept of scoring for classification of trauma patients is widely accepted today. Most of the standard scoring systems have been validated in Anglo-American settings. Because of differences in populations, differences in mechanisms of injury and differences in prehospital trauma care systems the question arose which score is valid and should be used in the Cologne trauma system.
A prospective trial testing seven standard scoring systems has been performed in Cologne from 01.01.1987 - 31.12.1987. 2.144 trauma patients were treated by emergency physicians in the field. A random sample of 625 patients were followed from the site of accident through their hospital course. Trauma Score (TS), Injury Severity Score (ISS), the TRISS-method, Revised Trauma Score (RTS), Glasgow Coma Scale (GCS), Prehospital Index (PHI) and the "Polytraumaschlüssel" (PTS) were calculated. The TRISS performed best predicting survival with a sensitivity of 93,1% and a specificity of 93.7% at a cut-off-point of 0.85. We conclude that TRISS is a valid tool for scoring trauma patients in the European setting. Quality assurance programs in trauma care should use TRISS to compare performance of different trauma systems.

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A POCKET-COMPUTER BASED PROGRAM FOR BEDSIDE-PRACTICABLE ASSESSMENT OF DISEASE SEVERITY AND SEPSIS EVALUATION IN INTENSIVE CARE MEDICINE. G. Pilz, T. Gurniak, O. Buidoso* and K. Werdan. Dept. of Medicine I, Grosshadern Munich Univ. Hospital and *Tropon Vertrieb Cutter, Cologne, Germany.

Despite their usefulness in quantitative evaluation in intensive care medicine, the use of scoring systems such as APACHE II (disease severity) and Elebute (sepsis evaluation) often has not become daily ICU routine. The lack of practicability (expenditure of time, knowledge of the score algorithm, necessity for undelayed score values availabil-

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ity) seems to be an important cause in raising the threshold for regular score monitoring. Thus, there is a need for a bedside-practicable, time-sparing and widely applicable device to enhance routine score calculation and data storage, with special regard to serial measurements (evaluation of disease progression in the individual patient). We present a pocket-computer based program written in BASIC that enables the ICU physician, even if unexperienced in scoring, to easily calculate APACHE II and Elebute score values within 5-10 minutes in a bedside manner. The program (38 kB) includes options for data outprint (patient's record) and storage (for transfer to personal computers).
Applications: APACHE II: assessment of disease severity and prognosis; evaluation of disease progression and of response to therapy; stratification of patient groups within clinical trials with regard to prognosis and severity of multiple organ failure; early prediction of patients at risk for post-operative complications (such as post cardiac surgery). Elebute: (calculation preceded, for practicability reasons, by a sepsis criteria screening): high specificity for the diagnosis of sepsis, especially in surgical patients; stratification of patients with regard to sepsis severity.
Conclusion: The presented program ensures a practicable and promptly available score assessment on the ICU for both clinical purposes as well as patients' inclusion and follow-up in clinical trials.

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APACHE II SCORE (Sc) IN THE EARLY DIAGNOSIS OF SEPTIC COMPLICATIONS (SeC) AFTER CARDIAC SURGERY (CS). S. Kääb, G. Pilz, E. Kreuzer* and K. Werdan. Depts. of Medicine I and of *Cardiac Surgery, Grosshadern Univ. Hospital, Univ. of Munich, Germany

In 110 non-selected patients (P) consecutively admitted to the ICU after CS (70 bypasses, 29 valve surgery, 11 miscellaneous, no heart transplantations), daily Sc monitoring was studied regarding its usefulness in the early diagnosis of SeC, a major cause of postoperative mortality. SeC (defined as Elebute sepsis score of ≥12 on ≥2 days) occurred in 16 P (SeP) and were associated with a significantly worse prognosis (mortality 69% vs 1%, p<0.0001) compared to non-SeP (NSeP).
Results: While preoperative Sc values revealed no difference between the 2 groups, significantly (p<0.001) higher Sc in SeP were noted already on the evening of the operation day ("0"). Furthermore, in contrast to NSeP who displayed a marked (p<0.001) fall in Sc from day 0 to day 1, SeP had unvarying high Sc:

Sc(x̄±SD) on days:		-1	0	1	2
SeP	(n=16)	12.8±2.7	22.1±4.8	21.4±6.7	21.3±9.6
NSeP	(n=94)	12.0±2.9	15.2±3.5	9.0±3.8	8.9±3.2

Compared to single parameters (fever, leucocytes), Sc proved to be superior in separating SeP and NSeP, showing already on the first postoperative day a maximal Youden index for SeC diagnosis (criterion: Sc ≥19) of 0.73 (vs. 0.33 for fever and 0.33 for leucocytes), with a sensitivity of 75% and specificity of 98% and predictive values (PV) of 86% (positive PV) and 96% (negative PV).
In a consecutive study carried out prospectively (independent group of 106 P), a Sc of ≥19 could be confirmed as discriminating criterion (mortality 36% vs 0%, p<0.0001).
Conclusion: In patients after cardiac surgery, the practicable APACHE II score seems to be a useful tool in the early diagnosis of patients at high-risk for developing postoperative septic complications.

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JAPAN COMA SCALE (JCS) IN THE EVALUATION OF IMPAIRED CONSCIOUSNESS IN THE ACUTE STAGE
T. Ohta and E. Takeuchi
Department of Neurosurgery, Osaka Medical School, 2-7 Daigaku-machi, Takatsuki-shi

The Japan Coma Scale (JCS) was established in Japan in 1974 for cooperative study of cerebral stroke. According to the JCS, conscious levels are initially divided into three stages: 1) awake, 2) can be awaken by stimuli, and 3) cannot be awaken even by stimuli. This classification is based on the grade of involvement of intracranial lesions toward the upper brain stem. The inter-observer reliability of the JCS was studied by unweighted kappa (κ), weighted kappa ($\kappa\omega1$, $\kappa\omega2$), and pi (π) methods. κ, $\kappa\omega1$, $\kappa\omega2$ and π values of the JCS for JCS-less well trained observers paired with JCS-well trained observers were 0.63 - 0.75, 0.84 - 0.90, 0.94 - 0.95, and 0.77 - 0.83, respectively. From the results that inter-observer reliability was high among observers who are well trained with the JCS but was lower among observers who are less trained with it, we speculated that this difference was due to the use of the term "arousal", the meaning of which is apparently ambiguous for the JCS-less trained observers. We therefore proposed a revision of the JCS in order to reduce the inter-observer variance by replacing the term "arousal" with more empirical criteria. We re-defined the term "arousal" used in the JCS as a combination of the eye opening, verbal, and motor responses. Further evaluation showed that the revised JCS had higher reliability than the original one even among JCS-less trained observers.

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S8: Endotoxin and Endotoxin Binding

MOLECULAR STRUCTURE OF ENDOTOXIN IN RELATION TO BIOACTIVITY 114

E.Th. Rietschel, L. Brade, H. Brade, H.-D. Flad, U.F. Schade, M. Schlaak, U. Seydel, A.J. Ulmer, P. Zabel, and U. Zähringer. Forschungsinstitut Borstel, D-2061 Borstel, FRG

Endotoxins are macromolecular amphiphiles and represent integral components of the outer membrane of Gram-negative bacteria. They play an important role in the pathogenesis and toxic manifestations of bacterial infection but are also involved in various physiological host-parasite interactions. Chemically, endotoxins are lipopolysaccharides (LPS) consisting of a polysaccharide portion and a lipid component, termed lipid A. The endotoxic activities of LPS are dependent on and mediated by lipid A. The primary structure of various lipid As is known. As an example, Escherichia coli lipid A consists of a 1,4'-bisphosphorylated β-D-glucosaminyl-(1-6)-α-D-glucosamine disaccharide which carries six fatty acids i.e. (R)-3-hydroxy- and (R)-3-acyloxyacyl residues in a defined distribution (1). E.coli lipid A has been chemically synthesized and shown to exhibit identical biological activity as its bacterial counterpart (ref. in 1,2). The endotoxic properties of lipid A depend on an unique primary structure and, thus, a peculiar conformation which is determined by the supramolecular arrangement of lipid A and the fluidity of its acyl chains (3). Endotoxic effects of LPS are promoted by host-derived mediators. Production and release of such mediators (TNF, Il-1, Il-6) were found to be induced by synthetic lipid A and endotoxin in human peripheral monocytes (4). In human volunteers, endotoxin caused significant elevation of serum levels of TNF and Il-6, the latter corresponding in time with a febrile response (5). In the regulation of endotoxin-induced formation of TNF and Il-6 the lipoxygenase product (S)-13-hydroxylinoleic acid appears to play an essential role (6).

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LPS, LPS BINDING PROTEIN, CD14 AND THE ACTIVATION OF MACROPHAGES BY LPS. 115

P.S.Tobias, J.C.Mathison and R.J.Ulevitch. Research Institute of Scripps Clinic, La Jolla, CA 92037

The activation of monocytes/macrophages (MO) in blood was recently shown to involve a tripartite complex of LPS, LPS binding protein (LBP) and CD14. LBP is a 60 kDa glycoprotein present in normal serum at <0.5 μg/ml whose concentration may rise 100-fold or more after an acute phase response. LBP binds to the lipid A moiety of rough and smooth form LPS with affinity in the nanomolar range. CD14 is a 55 kDa cell surface MO differentiation marker as defined by a variety of monoclonal antibodies (mAb) anchored to the plasma membrane via glycosylphosphatidylinositol. The net effect of the LBP/CD14 pathway is to markedly enhance the sensitivity and speed with which MO respond to LPS as compared with MO rsponses independent of the LBP/CD14 pathway. The functional properties of LBP and CD14 can be demonstrated both in particle adherence and in cytokine induction experiments. Thus LPS bearing particles such as gram negative bacteria or LPS coated erythrocytes are efficiently opsonized by LBP to bind to MO or CD14 coated surfaces; mAb to CD14 disrupt the binding. Similarly stimulation of MO to secrete TNF occurs at 100 fold lower [LPS] in the presence of LBP than in the absence of LBP. LBP. Finally, in whole blood, immunodepletion of LBP as well as blockade of CD14 with mAb can dramatically suppress TNF elicited by LPS. We postulate two pathways for MO activation by LPS; one involving CD14 and LBP, another as yet undefined. Further definition of these two pathways and their relationship will be discussed. Supported by AI25563, AI15136, GM28485, and GM37696 from the NIH.

CHARACTERIZATION OF ENDOTOXIN SPECIFIC RECEPTORS ON MAMMALIAN LYMPHORETICULAR CELLS. 116

D. C. Morrison*, M-G. Lei*, T-Y. Chen*, L. M. Flebbe*, J. Halling*, S. Field*, D. Dwyer* and S. Baykousheva*, Dept. Micro., U. Kansas Med Ctr, Kansas City, KS

Experiments from our laboratory using radioiodinated photoactivatable lipopolysaccharide (LPS) derivatives have allowed the identification of specific LPS binding proteins on the cell membranes of mouse and human lymphocytes, macrophages and other inflammatory mediator cells. One specific protein of approximate molecular mass 80 kDa has been extensively characterized and shown to have apparent binding specificity for lipid A. Hamster monoclonal antibody to this protein has been raised and shown to function as an agonist for LPS both in vitro, by the activation of mouse macrophages for tumor cell killing, and in vivo by initiation of enhanced transcription of Il-1 and TNF-α. Pretreatment of mice with monoclonal antibody to the 80 kDa LPS receptor has also been shown to protect animals against LPS lethality in the D-galactosamine sensitization model. Examination of LPS Receptor Distribution in mouse tissues using a monospecific rabbit anti receptor antibody indicate selective expression of LPS receptors on spleen, lymph node, lung, kidney and brain. Highest levels of expression were on endothelial cells and phagocytic cells (macrophages, Kupffer cells, mesangeal cells, astrocytes). More recent studies have suggested the existence of

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a second LPS binding protein of approximately 38 kDa on mouse and human lymphoreticular cells which appears to have binding specificity for KDO residues on LPS. Binding of E. coli S-LPS to this protein can be competitively inhibited in a dose dependent fashion by Re-LPS but not by purified lipid A. In contrast, as reported earlier, both lipid A and Re-LPS inhibit binding to the 80 kDa receptor. Current experiments are designed to investigate the functional role of this newly identified candidate KDO-specific LPS receptor as a second potential pathway for LPS dependent macrophage activation. (AI 23447).

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LPS INACTIVATION BY HUMAN SERUM LIPOPROTEINS AND PLASMA.

Willy A. Flegel and Hinnak Northoff. Abteilung für Transfusionmedizin, Universität Ulm, DRK Blutspendezentrale, D-7900 Ulm, Federal Republic of Germany.

Human lipoproteins inhibit various types of lipopolysaccharides (LPS) and lipid A from activating monocytes and from inducing release of monokines (IL1, TNF, IL6). Thereby, lipoproteins might control what is thought to be a major pathway of LPS effects in vivo.
Human plasma, anticoagulated by heparin or EDTA, could also be shown to inactivate LPS as measured by monokine release. Heparin in low, pharmacological doses caused a reduction in the overall LPS-inactivating capacity of plasma and serum. Whereas, low calcium concentrations facilitated LPS inactivation. Various serum properties (pH 5.0 - 9.5; temperature 32°C - 42°C) had only slight effects. Esterase inhibitors (like neostigmine) could not reduce LPS inactivation at all. Sera from hypercholesteremic patients treated with LDL (low density lipoprotein)-apheresis showed that LPS inactivation depended to a large extent on the LDL. The patients' sera before LDL-apheresis inactivated 5-fold more LPS than after removal of LDL. The LPS-inactivating capacity lost during apheresis could essentially be retrieved in the LDL-rich eluates of the apheresis columns. Direct comparison of lipoprotein classes prepared by ultracentrifugation confirmed that human LDL were more effective than HDL in inhibiting LPS from monocyte activation. So far, purified human apolipoprotein A1 (free of lipids) was also shown to inactivate LPS, indicating the importance of apolipoproteins in the described effects.
The findings support that the LPS-induced monocyte activation is controlled by a non-enzymatic interaction of LPS with lipoproteins. In humans, LDL are more effective than HDL. The results suggest that administration of heparin or Ca^{2+} should be considered as possibly reducing the efficiency of humoral mechanisms for detoxifying LPS.

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EVIDENCE OF IMMUNOMODULATORY FUNCTION OF SOLUBLE MONOCYTE ANTIGEN CD14.

C. Schütt, T. Schilling, C. Krüger
Dept. Medical Immunology, Ernst-Moritz-Arndt-University Greifswald, FRG

Membrane bound CD14 acts as a LPS receptor which recognizes LPS-LBP complexes. LPS-binding protein (LBP) is an acute phase reactant present in normal serum (WRIGHT, 1990). Alternative LPS induced monocyte activation mechanisms mediated by e.g. opsonizing antibodies are relevant for higher endotoxin concentrations. Independently from LPS concentrations (1 ng - 10 µg/ml) purified soluble CD14 molecules but not BSA or other proteins added to the opsonizing serum abrogate the oxidative burst response of monocytes to LPS in a dose dependent manner (2 - 30 µg/ml), measured by luminol enhanced chemoluminescence. Thus sCD14 is interfering with different endotoxins of E. coli and different opsonizing mechanisms and therefore a new candidate for shock prevention strategies. Soluble CD14 is present in normal human serum (4µg/ml ± 0.22, n = 102) measured by a capture ELISA. Elevation of sCD14 serum levels we observed in polytrauma, septicaemia or AIDS suggest an acute phase response for preventing endotoxin induced shock or organ failure due to monocyte mediator release.

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THE ENDOTOXIN-INDUCED PROCOAGULANT ACTIVITY OF MONOCYTES IS NOT INHIBITED BY LIPOPROTEINS.

E. Schlichting*, T. Henriksen*, T. Lyberg*.
Ullevaal Hospital, 0407 Oslo 4, Norway.

Toxicity of endotoxin is to a large extent mediated by the activation of monocytes/macrophages and the expression or release of monokines with a diversity of effects. Monocytes prepared from human blood and incubated with endotoxin in vitro, synthesize substantial amounts of thromboplastin the potent trigger of the extrinsic pathway of the coagulation system. The procoagulant response of monocytes is a highly sensitive marker of endotoxin effects, activating coagulation at the cellular level in doses which are 3-4 orders of magnitude less than what is required to activate the coagulation and other cascade systems of plasma.
Monocytes from healthy persons were stimulated with endotoxin (LPS from

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E-coli 026:B6) in doses 0.01-1 μg/ml. The thromboplastin activity was measured using a clotting assay and the activity activity increase (compared to non-stimulated monocytes) was 9.4 ± 1.9 -fold using the highest endotoxin dose.
Endotoxin binding to lipoproteins have been shown to diminish the endotoxin toxicity in vivo as well as in certain in vitro experimental settings. In our system the preincubation of endotoxin with different lipoproteins prepared by ultracentrifugation did not reduce the induced thromboplastin production. In fact, the VLDL fraction futher increased the thromboplastin-inducing capacity of endotoxin. The lipoproteins themselves had no inherent thromboplastin-inducing activity in monocytes.

ENDOTOXIN AND ENDOTOXIN-NEUTRALIZING-CAPACITY IN THE STATE OF NONSPECIFIC RESISTANCE AGAINST SEPTICEMIA FOLLOWING CECAL LIGATION AND PUNCTURE (CLP) OR WHOLE BODY IRRADIATION (WBI). **120**

Renate Urbaschek and K.-P. Becker* Institute Med. Microbiology & Hygiene, Klinikum Mannheim, University of Heidelberg, D-6800 Mannheim, Germany.

Induction of nonspecific resistance to bacterial infections can be induced by small amounts of endotoxins (ET) or their mediators, such as IL-1 or TNF. In the studies reported here, pretreatment with one single injection of 1 μg ET (from *E.coli* 0111, -24h or -48h) was used to study the state of enhanced nonspecific resistance against infections following CLP or WBI (825 cGy, ^{60}Cobalt) in NMRI mice. The lethality rates were 40% (controls 100%, $p \leq .01$) after CLP, and 10% (controls 100% in 15 days, $p \leq .001$) after WBI (observation time 30 days). Increased ET levels occurred 30' post CLP, at 6 h the values were reduced to 1 ± 1 in protected vs 31 ± 23 pg/ml in control mice. At this time the ET-neutralizing capacity in plasma (ENC, measured in our kinetic LAL microtiter test) was significantly increased in protected animals vs controls. - Extremely high ET values (>500 pg/ml) in plasma were measured on days 9 and 13 after WBI, whereas only slight increases in ET were detectable in protected animals. ENC was decreased at day 9 in both groups. Differences in body weight, aerobic and anaerobic bacterial CFU in blood and liver, peripheral blood, and femoral and splenic myelopoietic stem cell counts that occurred post-irradiation, will be presented. The data suggest that besides the known early recovery of hematopoiesis in the WBI model, the marked decrease in circulating ET may be related to the survival in the protected animals. In the more acute, severe septic shock after CLP, the increase in plasma ENC may be involved in the protective effect induced by pretreatment with ET.

BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN: A NATURALLY OCCURRING LIPOPOLYSACCHARIDE ANTAGONIST **121**

Marian N. Marra*, James L. Snable*, Randy W. Scott*, and Craig G. Wilde* (Spon: Charles J. Fisher Jr.)

Lipopolysaccharide (LPS) from Gram-negative bacteria is a potent stimulator of the inflammatory resonse. Overwhelming LPS challenge resulting from Gram-negative sepsis triggers overproduction of inflammatory mediators, such as tumor necrosis factor (TNF) from human leukocytes, which can mediate shock, multisystem organ failure and death. Bactericidal/permeability-increasing protein (BPI) is an antibiotic protein found in human neutrophil azurophil granules. We previously showed that purified soluble BPI isolated from human neutrophils binds LPS and inhibits LPS-mediated neutrophil and monocyte stimulation. Here we demonstrate that recombinant BPI binds LPS and that LPS binding can be inhibited by polymyxin B. Recombinant BPI also inhibits the release of TNF by human macrophages stimulated with LPS *in vitro*. Further, we show that BPI can be detected in supernatants of FMLP/cytochalasin B treated neutrophils, and is also present on the surface of LPS, TNF or FMLP stimulated neutrophils *in vitro*. Degranulation and release of BPI by neutrophils at the site of infection may represent a feedback mechanism by which neutrophils negatively regulate LPS-mediated induction of TNF. The exciting possibility that BPI may be a useful therapeutic agent to enhance the natural negative feedback mechanisms for regulating endotoxic shock is under investigation.

ANTAGONISM OF ENDOTOXIC LETHALITY AND CARBOHYDRATE DYSHOMEOSTASIS BY THE PROTEIN KINASE C INHIBITOR H-7. **122**

J. Filkins, J. Hunt*, and H. Inaba*.
Loyola Univ. Shock-Trauma Institute, Maywood, IL 60153

In vivo modulation of protein kinase C (PKC) by phorbol myristate acetate (PMA) augmented endotoxic lethality and sensitized to hypoglycemia and hyperlactacidemia (Amer.J.Physiol 260: #3, 1991). To test the specificity of PMA action and to evaluate the potential therapeutic efficacy of PKC inhibitors, we evaluated the effects of a

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potent and relatively specific PKC inhibitor H-7 [1-(5-isoquinolinesulfonyl)- 2 methylpiperazine dihydrochloride] on endotoxic lethality and alterations in plasma glucose and lactate levels using fed, male Holtzman rats treated with S.enteritidis lipopolysaccharide endotoxin (ETX).

		LETHALITY			PLASMA LEVELS 5HR	
GROUP	N	5hr	24hr	N	GLUCOSE mg/dl)	LACTATE (mM)
1. PMA + ETX	14	71%	93%	10	36±5.6	6.1±.82
2. PMA + ETX + H-7	14	21%	50%	10	87±12	2.8±.43
p value:		<.01	<.05		<.05	<.05

1. PMA at 0.5 mg/kg ip with ETX at 5 mg/kg iv.
2. H-7 at 25 mg/kg ip at 30 min prior to ETX and PMA.

Neither H-7 nor PMA alone altered plasma glucose or lactate levels at 5 hrs. ETX alone produced hyperglycemia without any lethality at 5 hrs and resulted in 25% lethality at 24 hrs. Therefore, the use of PKC inhibitors offers promise both to elucidate mechanisms in experimental models of endotoxicosis as well as to ameliorate the metabolic lesions and eventual lethality in severe septic states. (Supported by NIH Grant HL 31163)

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ENDOTOXIN-MEDIATED CHANGES IN PLASMA ENDOTHELIN CONCENTRATIONS, KIDNEY ENDOTHELIN RECEPTOR DENSITY AND RENAL FUNCTION IN RAT. P. Nambi, M. Pullen, M.J. Slivjak, B. Storer, M. Gellai, E.F. Smith III. SmithKline Beecham Pharmaceuticals, plc, Dept. of Pharmacology, King of Prussia, PA.

The purpose of these studies was to examine the relationships between endotoxemia and changes in plasma endothelin (ET-1) concentration, renal ET-1 receptor number and renal function in male Sprague-Dawley rats injected with nonlethal doses of 1 or 3 mg/kg LPS. Following the injection of 3 mg/kg LPS, plasma immunoreactive ET (irET), as measured by RIA, increased in a time-dependent manner: the maximal increase of 60% occurred at 6 hr after the injection of LPS ($p<0.05$), and thereafter returned to baseline levels. Prior to the injection of LPS, kidney ET-1 receptor density was 75±3 fmol/mg protein. At 6 hr after the injection of 1 or 3 mg/kg LPS, kidney ET-1 receptor density remained unchanged, whreas at 24 hr it was increased significantly by 70% in both groups ($p<0.001$). Scathard analysis confirmed the increase in kidney ET-1 receptor density, and suggested that there was no change in binding affinity (202 pM at baseline and 169 pM and 246 pM at 24 hr after the injection of 1 and 3 mg/kg LPS, respectively). At 7 d after the injection of LPS, kidney ET-1 receptor density was still increased by 30±5% and 58±16%, respectively ($p<0.01$; compared to the baseline value). Associated with the changes in ET-1 receptor number, there were significant decreases in Na^+, K^+, Cl^- and creatinine excretion. Baseline values for Na^+, K^+, Cl^- and creatinine excretion were 0.115±0.01 mmol/hr, 0.214±0.01 mmol/hr, 0.135±0.015 mmol/hr, and 8.01±0.3 mg/hr, respectively. At a dose of 3 mg/kg LPS, the maximal decreases in Na^+, K^+, Cl^- and creatinine occurred at 48 hr (-85%), 30 hr (-82%), 30 hr (-88%), and 30 hr (-33%), respectively. After 7 d, the effects of LPS on renal function were resolved. These results indicate that the increases in plasma irET concentrations preceded the changes in renal function, whereas the increase in renal ET-1 receptor density appeared concurrent to the impairment in electrolyte excretion. These studies suggest that ET-1, either by increases in plasma concentration or receptor number, may be involved in the LPS-induced impairment of renal function.

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EVIDENCE FOR *IN VIVO* ACTIVATION OF THE L-ARGININE/NITRIC OXIDE PATHWAY BY ENDOTOXIN G.A. Gray*, C. Schott*, I. Fleming*, J.R. Parratt [1] and J.C. Stoclet*
Lab de Pharmacol Cell et Molécul, Univ Louis Pasteur de Strasbourg, CNRS URA 600, 67401 Illkirch, FRANCE[1]Dept of Physiol & Pharmacol, Univ of Strathclyde, Glasgow, G1 1XW, U.K.

Recent evidence suggests that endotoxin (ETX) induces hyporeactivity to vasoactive agents through activation of a pathway producing a relaxing factor, probably nitric oxide (NO) from the amino acid L-arginine. The aim of the present study was to examine the role of this pathway in the hyporeactivity to noradrenaline (NA) induced by infusion of *E. coli* (O55:B5) ETX in anaesthetised rats. Infusion of ETX (10 mg kg^{-1} h^{-1}) had no effect on mean arterial blood pressure (MABP) but significantly decreased responses to NA (100ng - 1µg kg^{-1}). L-N^Gmonomethyl arginine (L-NMMA, 30 mg kg^{-1}) and L-N^{ω}nitro-arginine methyl ester (L-NAME, 1 mg kg^{-1}), specific inhibitors of NO synthesis from L-arginine, increased the MABP by approximately 30 mmHg and restored responsiveness to NA. Both effects were stereospecifically reversed by L- but not D-arginine. Restoration of responsiveness was not likely to be due to the increase in MABP alone since infusion of vasopressin to cause a similar hypertension did not inhibit the effects of ETX. Cyclooxygenase products of arachidonic acid have previously been implicated as mediators of ETX-induced hyporeactivity. However, in the present study neither the hypertension nor restoration of responsiveness by L-NAME were affected by indomethacin indicating that interaction with cyclooxygenase was not involved. The present results support the concept that NO is a major mediator of vascular hyporeactivity induced endotoxin *in vivo*.

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A L-ARGININE DERIVED RELAXING FACTOR IS INVOLVED IN THE ENDOTOXIN-INCLUDED HYPOREACTIVITY OF RESISTANCE VESSELS TO NOREPINEPHRINE. **125**

F. Schneider*, G. Julou-Schaeffer* and J.C.Stoclet* (Spon : J.R. Parratt)
Laboratoire de Pharmacologie Cellulaire et Moléculaire, Université Louis Pasteur de Strasbourg, URA CNRS 600, B.P. 24, 67401 ILLKIRCH, France

Loss of vascular responsiveness induced by endotoxin (ETX) in rats is counteracted by an inhibitor of NO production, both *in vivo* and *ex vivo* in the aorta (G. Julou-Schaeffer et al, Am. J. Physiol. 1990, 259 : H1038-H1043).We have now further investigated the effect of L-arginine (L-arg) and of the NO synthetase inhibitor L-nitroarginine methyl ester (L-NAME) on contractions induced by norepinephrine (NE) in resistance arteries (femoral and mesenteric arterioles) removed from rats 4 hours after injection of *E.coli* ETX (20 mgkg-1 ip). The arterioles were contracted with NE, in the presence or the absence of indomethacin (ind, 10 µM) and superoxide dismutase (SOD, 100 U.ml-1). The endothelium was either intact or rubbed, and its presence was assessed by the effect of acetylcholine (1 µM). Regardless of whether ind and the endothelium were present of not, the effect of NE was either not significantly affected or slightly depressed (in the presence of SOD) by ETX in arterioles, although aortic reactivity to NE was markedly impaired in the same rats (45 % cf decrease of maximal contraction). However L-(but not D-) arg induced relaxation of NE contracted arteries from ETX treated but not from control rats. The maximal effect of L-arg (1 mM) was larger in intact than in rubbed arteries and it was abolished by L-NAME (1 mM) and by the guanylate cyclase inhibitor methylene blue (10 µM). Our results show ETX induced the production of a nitric oxide like relaxing factor derived from L-arg not only in a conductance vessel but also in resistance arteries. They open new perspectives for understanding the mechanisms of circulatory failure induced by endotoxin.

DOES ENDOTOXIN MODIFY THE CELLULAR RESPONSE TO VASOCONSTRICTOR AGENTS IN CULTURED VASCULAR SMOOTH MUSCLE CELLS (SMC) ? **126**

G. Centeno, M. Burnier, B. Waeber, H.R. Brunner.
Div. Hypertension, Univ. Lausanne, CHUV, Lausanne, Switzerland

Depressed vascular reactivity to vasoconstrictor agents is a hallmark of septic shock. In animal models, the vascular responsiveness is suppressed within 45 min after exposure to endotoxin. The mechanism responsible for the loss of vascular reactivity is unknown. The present studies were conducted to evaluate the direct effects of endotoxin on cultured vascular SMC and the ability of endotoxin to modify the cellular calcium (Ca) response to angiotensin II (AngII). Primary cultures of rat aortic SMC were used. Changes in 45Ca uptake, 45Ca efflux and cytosolic Ca (Fura-2 AM) were determined in SMC exposed to 2 types of E. Coli endotoxin (O111:B4, Difco and O55:B5, Sigma) at doses ranging between 0.1 and 10 ug/ml. We also performed studies in which cells were stimulated with AngII (10-7 M) in presence or absence of endotoxin O111:B4 (0.1 to 10 ug/ml). Per se, both endotoxins had no immediate effect on cellular Ca metabolism in these aortic SMC except for a slight increase in 45Ca uptake at very high concentrations (100 ug/ml). Stimulation with AngII produced significant increases in Ca uptake, Ca efflux and cytosolic Ca . These AngII-induced Ca changes were similar in presence of endotoxin . Moreover, AngII binding to its receptor was comparable in SMC incubated for 1 h with endotoxin O111:B4 or its vehicle. These results show that endotoxin has no direct effect on cellular Ca metabolism in aortic SMC and does modify neither the cellular response to AngII nor its binding to the receptor. They would therefore suggest that the loss of vascular responsiveness is unlikely due to a direct effect of endotoxin on vascular SMC .

ENDOTOXIN DIRECTLY STIMULATES THE PRODUCTION OF AN L-ARGININE DERIVED RELAXING FACTOR IN VASCULATURE SMOOTH MUSCLE VIA A PROTEIN SYNTHESIS DEPENDENT STEP. **127**

L. Fleming*, G. A. Gray*, J.R. Parratt[1] and J.-C. Stoclet*.
Laboratoire de Pharmacologie Cellulaire et Moléculaire, Université Louis Pasteur de Strasbourg. 74 Route du Rhin, 67401 Illkirch, France and [1]Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW Scotland.

Endotoxin (ETX)-induced hyporesponsiveness to noradrenaline (NA) was investigated by incubating endothelium denuded rat aortic rings with ETX (100 ng ml^{-1}) for 1 to 5 h. Contractile responsiveness to NA (1 nM to 10 µM) was significantly depressed after 3 h incubation, an effect which increased with the incubation time. Inclusion of indomethacin (10 µM) during incubation was without effect but expression of the phenomenon was completely inhibited when cycloheximide (100 µg ml^{-1}) was present throughout the experiment. Hyporesponsiveness to NA (1 µM) reappeared 3 h after removal of cycloheximide, was reversed by L-NMMA and L-NAME (inhibitors of the L-arginine pathway) and by methylene blue (inhibitor of soluble guanylate cyclase). Addition of L-arginine (1 mM), further depressed reactivity to NA. Furthermore levels of cyclic GMP were found to be 4 fold greater in ETX-treated media than in control media. These results indicate that ETX-induced hyporeactivity

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(1) can result from a direct interaction between ETX and the vasculature (2) requires a time lag of approximately 3 h (3) is independent of cyclooxygenase products (4) involves de novo protein synthesis (5) involves activation of the L-arginine pathway in smooth muscle cells and is mediated by the subsequent elevation in the tissue cyclic GMP content.
Supported by the Commission of the European Communities (Grant N° ST2J-0457-6).

128 IMPLICATIONS OF NITRIC OXIDE FORMATION DURING PORCINE ENDOTOXIN SHOCK

M. Cirino, C. Motz and A.W. Ford-Hutchinson (Spon: R. C-J. Chiu)
Merck Frosst Centre for Therapeutic Research, Dept. of Pharmacology, Kirkland, Québec, Canada H9H 3L1

Inhibition of nitric oxide (NO) synthesis from endogenous L-arginine by N^{ω}-nitro-L-arginine methyl ester (**L-NAME**) leads to vasoconstriction and systemic hypertension. Bovine aortic endothelial cells incubated with *E. coli* lipopolysaccharide (**LPS**) release a NO-like factor. In pigs given LPS severe cardiopulmonary alterations occur including a marked increase in pulmonary vascular resistance and profound reductions in mean systemic arterial blood pressure (**MAP**). To investigate whether NO formation might account for this hypotension, anesthetized pigs (sodium pentobarbital, 20 mg/kg,iv) were treated with L-NAME before and after the administration of LPS (1.0µg/kg,iv). Injection of L-NAME (1.0mg/kg,iv) produced a sustained 47% increase in MAP, however, this pretreatment did not prevent or ameliorate either the initial or late hypotensive responses characteristic of endotoxic shock in pigs. The second marked decrease in MAP, which occurs approximately 3 hours after the administration of LPS, to date, has not been associated with any known vasodilating agent. L-NAME administered during this period increased the MAP from a low of 62±3 mmHg to 90±12 mmHg. As well, there was an augmentation of the pulmonary arterial pressure from 23±4 mmHg to 34±3 mmHg and a modest (18%) reduction in cardiac output. The effects of L-NAME were reversed by the administration of L-arginine. These results indicate that NO formation plays a role in the second and often fatal component of cardiovascular collapse that follows LPS administration.

129 DIFFERENCES BETWEEN ENDOTOXIN AND SODIUM NITROPRUSSIDE IN IMPAIRING VASCULAR REACTIVITY.

M.O. Guc*, B.L. Furman and J.R. Parratt.
Department of Physiology & Pharmacology, Strathclyde University, Glasgow G11XW, Scotland, U.K.

Endotoxin impairs vascular reactivity, at least in part, by increasing the release of nitric oxide, which increases cyclic GMP in vascular smooth muscle (Fleming et al 1990). The present work was undertaken to determine if the effects of endotoxin on vascular reactivity are mimicked by sodium nitroprusside (SNP), which also produces vasodilatation via cGMP generation. Studies were performed in pithed rats, ventilated with 100% O_2 and infused with *E. coli* endotoxin (250 $\mu g\ kg^{-1}h^{-1}$), SNP (400 $\mu g\ kg^{-1}h^{-1}$) or saline. Dose-response curves to various vasoconstrictors were constructed at 1h into the infusions. Endotoxin or SNP each produced significant hypotension at 1h (control, mmHg 58±1, endotoxin 47±1, SNP 42±2). Vasopressor responses to cirazoline (α_1 agonist), endothelin and 5-HT were impaired by SNP, whereas the responses to BHT 933 (α_2 agonist) were unaffected. For example the E_{max50} value (µg/kg) for cirazoline was increased from 3.5±0.5 to 25.2±5.2 ($P<0.05$). Endotoxin similarly impaired responsiveness to cirazoline and 5-HT. In contrast to SNP, endotoxin reduced responsiveness to BHT 933 but did not modify that to endothelin. Similarly to endotoxin, the impairment of responsiveness to vasopressor agents produced by SNP was prevented if SNP-induced hypotension wae prevented by co-infusion of vasopressin (0.64 i.u. $kg^{-1}h^{-1}$). Although much evidence supports the role of nitric oxide in endotoxin-induced impairment of vascular reactivity, the differences between endotoxin and sodium nitroprusside may suggest that additional factors are involved.

Fleming,I. et al.(1990) Biochem. Biophys. Res. Comm. 171:562-568.

130 ENDOTOXIN INDUCED ALTERATIONS IN ATRIAL NATRIURETIC FACTOR.

P. Rao, D. Cavanagh J. Florica*, and L. Graham*. Dept. of OB/GYN, University of South Florida, Tampa, FL 33612.

Gram-negative sepsis is often associated with renal failure. Atrial natriuretic factor (ANF) is thought to be important in volume homeostasis by virtue of its diuretic, natriuretic and vasodilatory properties. To our knowledge the circulating levels of ANF in this condition has not been reported and needs to be addressed. Anesthetized dogs were studied, with one group receiving isotonic saline (C,n=6), while the other group received 0.05 mg/kg of endotoxin intravenously (E, n=7) over a 4 hour period. Mean values (± SEM) for arterial pressure (AP), renal artery flow (RAF), creatinine clearance (C_{cr}), urine output (UO), and ANF are presented as baseline value and at hourly intervals thereafter.

Notes

	Baseline		1 hr		2 hr		3 hr		4 hr	
	C	E	C	E	C	E	C	E	C	E
AP(mmHg)	102±7	97±4	99±6	93±8	102±8	83±6	103±7	85±7	110±6	104±4
RAF(ml/min)	149±20	151±16	168±24	181±34	186±30	170±23	186±24	151±27	176±23	152±16
C_{cr}(ml/min)	36±5	35±5	43±5	49±10	43±5	32±6	42±9	25±6	38±6	25±2
UO(ml/min)	.13±.02	.19±.03	.21±.03	.39±.09	.32±.05	.33±.07	.33±.06	.18±.06	.34±.08	.30±.06
ANF(fmol/ml)	14.4±1.4	15.8±1.8	12.1±1.7	19.1±1.1	12.4±.94	22.2±4	12.3±.4	24.3±4	11.6±1.4	25.2±2.8

Significant differences ($b < 0.05$) occurred, both within and among the groups at different time intervals. Serum sodium, potassium and osmolalities however, have remained relatively stable. This study suggests that a progressive increase in circulating ANF occurs with the continuous infusion of a small dose of endotoxin. Despite a modest decrease in creatinine clearance, urinary output was well maintained with some variability being observed in AP and RAF. While it is reasonable to suggest that ANF participates in modulating renal function under our experimental conditions, other hormonal interactions should also be considered.

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CONTRIBUTION OF NEUTROPHILS TO LETHALITY DURING SHOCK INDUCED BY LIPOPOLYSACCHARIDE.
J. Barroso-Aranda, G. W. Schmid-Schönbein, B. W. Zweifach, and J. C. Mathison*, Univ.of California, San Diego, La Jolla, CA, 92093-0412, USA and *Research Institute of Scripps Clinic, La Jolla, CA 92093, USA

Our objective was to study mechanisms by which neutrophils (PMNs) produce lethality after injections of lipopolysaccharide (LPS). For this purpose control rats were compared with LPS-tolerant rats. Tolerance was induced by daily ip. injections of sublethal doses of LPS for 4 days. Both groups were subjected to 9 mg/kg *E. coli* LPS iv. which resulted in 25% survival in LPS-controls vs. 100% in LPS-tolerants. LPS injection caused a rapid neutropenia in both groups, which remained throughout the experiment in controls. In contrast, in LPS-tolerants there was a conspicuous increase in the PMN count reaching after 6 hrs values 16-fold higher than controls. PMN activation, measured by nitroblue tetrazolium reduction, was lower in LPS-tolerant animals. PMN adhesion to nylon fiber columns was dramatically lower in LPS-tolerants. Measurements of tumor necrosis factor (TNF)/cachetin activity shows high values in controls and no detectable TNF activity in LPS-tolerant group. The degree of microvascular obstruction by PMNs in the heart, measured from histological sections, was limited to true capillaries with a higher percentage in LPS-controls than LPS-tolerants. We conclude that PMNs play an important role in LPS mediated shock. LPS pretreatment reduces adhesion and activation of PMNs, resulting in high circulating counts and low degree of microvascular trapping. (Supported by USPHS Grant HL10881, Hl43026, and Hoechst-Roussel Pharmaceuticals, Inc.).

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METALLOTHIONEIN GENE EXPRESSION IN RAT LIVER DURING ENDOTOXIN SHOCK, AS REVEALED BY IN SITU HYBRIDIZATION.
N. Taga, T. Takahashi, Y. Itano, A. Nagai, H. Hidaka, S. Noji, S. Taniguchi, and F. Kosaka (Spon: K. Okada). Dept. of Anesthesiology and Resuscitology, Okayama Univ. Med. Sch. and Dept. of Biochemistry, Okayama Univ. Dental Sch. Okayama, 700, Japan

Metallothioneins(MTs) are zinc-binding proteins of low molecular weight and known to be induced in the liver during endotoxin shock as an acute phase reactant. It has been reported that their functions include detoxification of heavy metals, metabolism of trace elements, and scavenging of free radicals. In the present study, in order to further clarify the significance of induction of hepatic MTs during endotoxin shock, we investigated the expression of MT genes, as typical acute-phase genes, in bacterial endotoxin-treated rat livers by in situ hybridization method with riboprobes synthesized from mouse cDNA of the MT-II. We found that the MT genes are expressed intensively and specifically in the periportal area of the liver lobule. On the other hand, MT gene expression was weak around the central hepatic vein of the liver lobule. In H.E. stained section, hypoxic liver cell damage which appears to be the result of endotoxin shock was observed around the central vein, whereas liver cells of the periportal area was relatively intact. These results indicate that MTs may be involved in the protection of the liver cells by scavenging the free radicals which is considered to be one of the cause of liver cell damage during endotoxin shock.

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ENDOTOXIN-INDUCED ELEVATIONS OF PLASMA CALCITONIN GENE-RELATED PEPTIDE (CGRP) LEVELS DEVELOP TOLERANCE AFTER MULTIPLE EXPOSURES TO LOW-DOSE ENDOTOXIN.
R.R. Fiscus*, X. Wang*, Z. Zhou*, M. Oi* and S.B. Jones. Sanders-Brown Research Center on Aging and Department of Physiology & Biophysics, University of Kentucky College of Medicine, Lexington, KY 40536-0230, and Department of Physiology, Loyola University Medical Center, Maywood, IL 60153. USA

Our previous studies suggest that CGRP plays an important role as a mediator of hypotension in both early and late stages of endotoxin shock as well as in late stages of hemorrhagic and septic shock. CGRP is an

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extremely potent vasodilator, found co-localized with substance P in nociceptive sensory nerves innervating blood vessels and many other organs. Because inflammatory mediators, such as bradykinin, histamine and prostaglandins, are known to activate these nerves and cause local release of CGRP and substance P, these inflammatory mediators may play a critical role in CGRP release during endotoxicosis. Supporting this idea, we have shown that anti-inflammatory agents, dexamethasone, ibuprofen and indomethacin, block endotoxin-induced release of CGRP. Because generation of prostaglandins and hypotensive responses to endotoxin develop tolerance (i.e. become reduced) following multiple exposures to low doses of endotoxin, we determined whether endotoxin-induced release of CGRP may also develop tolerance. To produce tolerance, rats were injected each day for 4 days with endotoxin (lipopolysaccharide B from *S. Enteritidis*) at gradually increasing doses (0.1, 0.5, 1.0 and 2.0 mg/kg, i.p.). On 5th day, rats were anesthetized and left carotid artery and right jugular vein were cannulated for later measurements of arterial pressure, withdrawals of arterial blood for CGRP assays, and intravenous (i.v.) injections of endotoxin. Two days later, endotoxin (5 mg/kg, i.v.) was administered to control rats (cannulated, but not previously injected with endotoxin) or endotoxin tolerant rats. In control rats, endotoxin lowered arterial mean pressure by 30 mm Hg at 30 and 60 min and elevated plasma CGRP levels (4.11 ± 0.86 to 30.8 ± 4.4 pg/ml) at 3 hr. Endotoxin also decreased glucose levels (150 ± 16 to 107 ± 12 mg/dl) and increased lactate levels (1.15 ± 0.12 to 4.78 ± 0.56 mM) in plasma of control rats at 3 hr. In rats made tolerant, all responses to endotoxin, including plasma CGRP elevations (4.72 ± 1.59 pg/ml), were either absent or greatly reduced. The data indicate that endotoxin-induced release of CGRP, like hypotension, hypoglycemia and hyperlactacidemia, develop tolerance following multiple exposures to increasing, low doses of endotoxin. The data thus provide further support for the hypothesis that CGRP, released by intermediate inflammatory mediators (eg. prostaglandins), plays an important role as a final extracellular mediator of the hypotension during the pathogenesis of endotoxin shock.

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FIBRIN-MONOMERS IN PROLONGED EXPERIMENTAL ENDOTOXIN INDUCED SHOCK IN PIGS. Chr. Agternkamp*, E. Beeke*, E. Loxtermann*, R. Lade*, F.G. Müller*, G. Kalff* (Spon: H. Redl).
Clinic for Anesthesia, Aachen Univ. of Technolog., Pauwelsstr. 30, D-5100 Aachen, Germany

The impact of fibrin-monomers in disseminated intravascular coagulation (DIC) has been a topic of research in recent years. The specific aim of this study was to show the massive effects of septic processes to the coagulative system by directly measuring the concentration of fibrinmonomers in plasma.

In our study we created a septic shock in 25 female minimal disease pigs using E-coli B4:0111 LPS W (Fa. Difco) endotoxin up to 4 ug/kg KG/h. This state of shock was prolonged by only a "suboptimal treatment" up to 96 hours. According to a fixed schedule, immediately before the first endotoxin infusion was administered, after one, three and then every four hours, blood samples were taken, in which the concentrations of fibrin-monomers were measured by a spectrophotometric assay.

Using the same scheme we also measured the concentration of fibrin-monomers in pigs without any endotoxin applications. Our study shows the extreme increase in fibrin-monomer concentration (more than 300 %) in endotoxin treated pigs as compared to the control group, without any significant change. This spectrophotometric assay for the determination of fibrinmonomer concentration in plasma is a simple method for discovering and observing the cource of DICs. By differentiating easily between hypo- and hypercoagulative states we are now able to interfere earlier in DICs which are common in septic proccesses.

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REDISTRIBUTION OF CARDIAC OUTPUT IN PAF (PLATELET ACTIVATING FACTOR) OR ENDOTOXIN INDUCED SHOCK. M.F. Mulder, A.A. van Lambalgen, A.A. van Kraats, B.J. Appelmelk, G.C. van den Bos, L.G. Thijs. Lab. for Physiology and Depts. of Medical Microbiology and Internal Medicine, Free University, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

PAF may be involved as a mediator in the pathogenesis of endotoxin shock. To assess its possible role in the redistribution of cardiac output during endotoxin shock we have compared the effects of PAF and endotoxin infusion. Endotoxin (*E.coli* O127:B8, 8 mg/kg.h; E-group, n=7) or PAF (3 µg/kg.h; P-group, n=7) was infused into male anesthetized (pentobarbital) Wistar rats for one hour to cause a same decrease in cardiac output (by circa 40%) in both groups. Cardiac output (CO), organ blood flow as percentage of CO (%CO; radio-active microspheres), mean arterial pressure and heart rate were measured before and after the infusion. At t = 60 min decrease in CO was not significantly different for E- (-39%) and P-group (-44%). Heart rate had increased in both groups (+10% in E- and +6% in P-group; $p<0.05$). Mean arterial pressure decreased more ($p<0.05$) in the P- (-55%) than in the E-group (-32%). In the E- and P-group %CO to heart increased (by 99 and 86%, resp.), to skin decreased (by 57 and 39%, resp.), while it did not change to kidney, skeletal muscle and brain. In the E-group %CO to pancreas, stomach, small intestine, colon and adrenals fell (by 30, 42, 24, 8 and 50%, resp., $p<0.05$) whereas there was no change in the P-group (except for an increase by 59% to pancreas). %CO to hepatic artery and diaphragm increased in the E- (by 98 and 122%, resp., $p<0.05$) but did not change in the P-group. In spleen and trachea %CO decreased in the P- (by 58 and 61%, resp.) but did not change in the E-group. Redistribution of CO in PAF- and endotoxin-treated rats was thus not the same: in the gastro-intestinal tract and pancreas PAF seemed to cause vasodilation and endotoxin vasoconstriction. In conclusion other mediators than PAF must also be involved in the redistribution of cardiac output in endotoxin shock.

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THE EFFECT OF LIPID A ON RES. **136**

R. Karim, N. Freudenberg, CH. Vogt, M.A. Freudenberg and C. Galanos.
Chir. Klinik Köln Holweide, Patholog. Institut Univ. Freiburg i. Br. and Max-Planck-Institut Freiburg i. Br.

In animal experiment time and dose depending effects of Lipid A to phagocytic activity of the RES, to the DNA synthesis of sinusides of the liver, hepatocytes and to the GPT activity were explored. A toxic and a nontoxic dosage of Lipid A was applicated.
During the first hour after Lipid A application in both toxic and nontoxic doses a depression of phagocytic activity was observed. As a reason of the decreased phagocytic capacity not only a damage of the sinusides in the liver and an overlaod of these with Lipid A but also an increase of metabolic products by shock should be expected. During the following days after injection of the shock inducing amount of Lipid A the phagocytic activity in RES increased and reached it s maximum on the fifth day. This effect could not be observed after the application of the nontoxic dose.
The rate of DNA synthesis of the sinusides in the liver was obviously augmented in both dosages on the third day after application. After 14 days the standard rate was reached again. The initial higher increase of the DNA synthesis after the high dose of Lipid A was considered to be a repair process after the shock inducing damage of this substance. We discuss the gain in DNA synthesis following the application of lower dosage Lipid A as a mitogenous effect.
The poor increase of the GPT activity and the DNA synthesis of the hepatocytes may be explained as response of hepatocytes to gentle damage by Lipid A.

THE CHANGE OF ERYTHROCYTE CALCIUM IS ONE OF THE MECHANISMS OF ANAEMIA FORMATION IN RABBIT WITH ENDOTOXIN-INDUCED DIC. Q.Wu, Y.Wang, J.Tao and Y.Dang (Spon:NSFC and CMB) Institute of Basic Medical Sciences, PUMC and CAMS, 100730, Beijing, China **137**

The effects of E. coli endotoxin (ET) on human erythrocyte cytosolic free calcium concentration (Ca^{2+})i and activity of membrane calcium pump (Ca-Mg ATPase) were observed in vitro. A disseminated intravascular coagulation (DIC) model was prepared on rabbits by intravenous injection of ET two times. The (Ca^{2+})i was measured by fluoremitry with indicator fura-2. The (Ca^{2+})i of human erythrocyte was 86±9.2 n mol in normal and it was increased to 124±22 n mol, 174±41 n mol 220±92.1 n mol respectively when the erythrocyte were incubated with ET in 0.5 mg/ml, 1 mg/ml and 2 mg/ml. However, the Ca-Mg ATPase activities decreased from 1031±131 n mol/mg.h in normal to 870±182 n mol/mg.h , 684±124 n mol/mg.h and 718±144 n mol/mg.h in grups of ET. The(Ca^{2+})i of rabbit erythrocyte was elevated from 76.6±4.9 n mol in normal to 224±74 n mol and the Ca-Mg ATPase activity was inhibited from 720±26 8 n mol/mg.h in normal to 146.1±30.6 n mol/mg h during ET-induced DIC in vivo. In addition, the erythrocyte deformability decreased and the membrane protein electrophoresis in band 4.5 area changed, however there was no significant difference in the calmodulin activity, fluidities of membrane lipids and sulfhydrylprotein of erythrocytes in DIC rabbits.The results obtained suggest that there is a possible alternative mechanism of the microangiopathic haemolytic anaemia formation in ET-induced DIC of rabbit It seems to be that ET could damage the cell membrane, inhibite calcium pump activity and increase the cytosolic free calcium concentration which initiate a series of calcium-dependent proteinase and phospholipase, and result in the membrane injury further On this condition, the erythrocytes become "prolytic" and were schismatic easily by blood flow.

Plenary Session: Sepsis and Organ Failure

INFLAMMATION AND MOF - THE HORROR AUTOTOXICUS. Arthur E. Baue. St. Louis University Medical Center, St. Louis, MO 63110-0250, USA. **138**

Paul Ehrlich, in 1901, wrote of the "Horror Autotoxicus" the destruction which might be brought about by an autoimmune reaction. Today we have learned that patients after operation or injury may be threatened by the mechanisms and mediators that should protect them. An inflammatory reaction, necessary for survival, if overwhelming can lead to MOF. Infection and/or severe inflammation underlie the development of MOF, but blocking or interfering with the mediators of the response may be deleterious. Replacing lost factors, such as surfactant, controlling infection by host defense support and growth factors may be helpful. In the meanwhile, much can be done to prevent MOF. Principles of prevention include: 1. improving microcirculatory blood flow early to decrease ischemia-reperfusion injury (rapid, warm volume replacement, hypertonic solutions, ATP-$MgCl_2$, pentoxifylline). 2. Controlling the injury by early definitive operation and fracture fixation. 3. Removing as much necrotic tissue as possible as soon as possible. 4. Promoting a metabolism by enteral nutrition, gut support and specific nutrients. 6. Prevent infection by maintaining an adequate immune response with growth factors

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or other immunostimulants, appropriate antibiotics and wound and body cavity care. 7. Treating infection early and adequately when it develops by operation, monoclonal antibodies to endotoxin and other agents. These approaches along with a better understanding of the hypermetabolic, inflammatory, septic state and its biologic mechanisms should best prevent MOF.

139 SYSTEMIC HOST-DEFENSE FAILURE DISEASE CAUSED BY POST-TRAUMA AND SEPTIC DEREGULATION OF THE EICOSANOID-CYTOKINE-HEPATIC ACUTE PHASE PROTEIN RESPONSE TO INJURY. John H. Siegel, MIEMSS, Univ. Maryland, Baltimore, MD 21201

Traumatic injury or invasion by infectious organisms initiates an organized systemic host-defense response involving a primary eicosanoid-cytokine-leukocyte **inflammatory response** and a secondary **solid organ metabolic response** in which hepatic acute phase protein synthesis serves as a modulating feedback regulator of the inflammatory reaction. The acute inflammatory response is initiated at the site of injury or septic invasion and is made systemic by the medium of the circulating blood, which carries these injury mediators throughout the body to induce an increased microvascular permeability of the capillary endothelium. As a result cytokine mediators initiated by the acute inflammatory phase leave the localized area and are brought to distant areas of altered capillary permeability and pass to the interstitial fluid - cell membrane interfaces of remote organs to initiate the solid organ metabolic response. Here they act on muscle and liver cells to alter the interorgan substrate-energy flux to change the normal relations between protein synthesis and protein degradation. The net effect is to reprioritize and shift protein synthesis from the organs of locomotion to the organs of host-defense which include the immune system and the liver. The resulting hepatocyte response which produces the acute phase proteins serves to enhance certain aspects of the inflammatory process while modulating others. When the host-defense response process becomes disordered by excessive cytokine elaboration, a sequence of **autodestruction of both cells and organs occurs. This autodestructive Host-Defense Failure** disease induces the Multiple, or Sequential Organ Failure Syndromes.

141 GUT AS A SOURCE OF MOF. Edwin Deitch, LSU-Medical Center, Department of Surgery, Shreveport, Louisiana, U.S.A.

Traditionally, evaluation of intestinal function has been limited largely to monitoring gastric pH and intestinal motility. This clinical approach has led clinicians to equate normal intestinal motility with normal intestinal function and to assume that if stress-induced gastric bleeding can be prevented, all will be well. However, it is becoming increasingly clear that the gastrointestinal tract is not a passive organ and that intestinal dysfunction is not limited to ileus and upper gastrointestinal bleeding. Instead, the gastrointestinal tract is recognized as having important endocrine, metabolic, immunologic, and barrier functions, as well as its traditional role in nutrient absorption. Over the last 10 years, there has been a resurgence of interest in the role of intestinal barrier failure in the critically ill or injured patient. This review will present the results of human and animal studies on the role of intestinal barrier failure and bacterial translocation as a promoter or potentiator of systemic sepsis and MOF.

142 **SEPSIS AND MULTIPLE ORGAN FAILURE.** W.J. Sibbald, *University of Western Ontario, London, Ontario, Canada.*

Multiple organ failure (MOF) is often the decisive event determining outcome in critically-ill patients. Common antecedents include multiple trauma, pancreatitis, burns, severe infection and shock. Common to these events is activation of the host's inflammatory system, a process which involves cell-cell interaction, the production of cytokines, neutrophil activation, endothelial damage and diffuse pulmonary and nonpulmonary organ injury (Table 1). It has been proposed that a process of "malignant inflammation" characterizes the development of MOF, and is due to excess production of inflammatory mediators and/or failure of the host to control the normal sequelae of inflammation.

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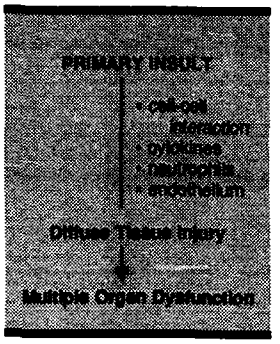

An important paradox is that the processes causing diffuse pulmonary and extrapulmonary organ injury exhibit the capacity to be self-perpetuating. *Circulatory dysfunction* accompanying MOF may lead to a mismatching of tissue oxygen needs versus availability, and tissue ischemic injury may result. *Gastrointestinal dysfunction* may propogate MOF by two mechanisms. First, the translocation of endotoxin and bacteria across a permeable barrier membrane may induce further tissue injury via the direct and remote effects of endotoxin and/or the sequelae of repeated bacteremias; this may be particularly evident in the presence of *Hepatic dysfunction*. Second, nosocomial pneumonia may complicate the aspiration of gram negative aerobic bacilli which colonize the gut in critical illness. All of *Renal dysfunction, Respiratory dysfunction,* and *Central Nervous System dysfunction* establish an obligate need for life-support technology, a process which further promotes the development of nosocomial infections and thereby augments the heightened inflammatory response remote from the primary insult.

Major advances in *Treatment* must focus on *prevention*, and must emphasize *cost-effectiveness*. Measures to maintain the integrity of the gastrointestinal tract in "at-risk" patients include *early enteral feeding and selective gut decontamination*. *Active treatment* of septic patients with immunotherapeutic agents, ie. monoclonal antibody to endotoxin, may further reduce the incidence of MOF in critically-ill patients.

VASCULAR FAILURE IN SEPTIC SHOCK. L.G. Thijs, A.B.J. Groeneveld. Medical Intensive Care Unit, Free University Hospital, 1081 HV Amsterdam

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Septic shock is initiated by a sharp fall in the systemic vascular resistance (SVR). Peripheral vascular dilatation may reach the point where loss of regulatory function of the arteriolar vasculature results in dependency of blood pressure on cardiac output in particular in nonsurvivors. Nonsurvivors appear not to be able to regulate SVR to a fall in cardiac output. This phenomenon may contribute to mortality, as a common denominator of patients who die of septic shock is persistent vasodilation, clinically characterized by unrelenting hypotension and marked loss of reactivity to vasoconstrictors. In addition to other mechanisms in the peripheral circulation such as microembolization and endothelial cell injury, inappropriate vasodilation contributes to maldistributed microcirculatory flow with relative under- and overperfusion in relation to tissue needs. This phencmenon may be related to ineffective peripheral oxygen extraction as SVR is often correlated with oxygen extraction ratio and a fall in SVR may be associated with a rise in arterial blood lactate levels (and vice versa) as a rough marker of O_2 supply/demand imbalance. Microcirculatory failure is a basic mechanism in septic shock associated with tissue ischemia. In the heart in canine endotoxin shock in fact evidence for maldistributed flow in the presence of a normal global perfusion has been obtained. A large number of mechanisms and mediators are suggested to be operative in inducing these microcirculatory abnormalities but recently an essential role for nitric oxide in inducing vasodilation and diminished responsiveness to adrenergic agents has been suggested.

S9: Neuro-Endocrinological Systems

ENDOCRINES, CYTOKINES AND SHOCK. John W. Holaday, *Medicis Pharmaceutical Corporation, 100 E. 42nd St., New York, NY 10017 U.S.A.*

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Over the past decade, it has become evident that the neuroendocrine system and immune system are functionally linked; the body's orchestrated responses to trauma, shock and sepsis involve all aspects of this neuroendocrine-immune axis. Furthermore, many of the drugs commonly used in the management of patients suffering from circulatory shock profoundly affect host defenses by changing the neuroendocrine milieu. Although the immunosuppressive effects of endogenous or pharmacologically administered glucocorticoids have been known for some time, the immunological importance of other neuroendocrine hormones such as adrenocorticotrophic hormone (ACTH), endorphins, prolactin and growth hormone have only recently been recognized.

The profound physiological stress of endotoxemia and septic shock has long been known to affect the release of many pituitary hormones; it has been assumed that this hormonal response to shock was mediated by the classic neuroendocrine circuitry. In this symposium, I will present data to indicate that endotoxin may cause profound changes in neuroendocrine hormones by causing the release of monokines such as IL-1 and TNF that, in turn, act directly and indirectly on the neuroendocrine axis. The spectrum of hormones released by either stress or by the action of monokines may then have further effects on host defenses by mediating subsequent immunological processes. Specifically, ACTH releases adrenal glucocorticoids in sufficient concentrations to alter the immunological cascade, including lymphocyte release of monokines and cytokines. Prolactin appears to potentiate T-cell proliferation and thereby stimulate immune responses, and Growth Hormone appears to be involved in the expression of cytokines from monocytes. As one additional level of complexity, the pioneering work of Blalock and colleagues has demonstrated that many "pituitary hormones" are found in lymphocytes; we have recently demonstrated that lymphocytes contain a prolactin-like protein (PLP) with a molecular weight of 48 Kd that is increased several fold by mitogens and may function as an autocrine cytokine that facilitates lymphocyte proliferation in response to endotoxin and other mitogens.

I thank my co-authors, Drs. E. Bernton, H. Bryant, P. Smith and J. Kenner.

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HYDRAZINE SULFATE: A NOVEL PROTECTIVE AGENT AGAINST THE LETHAL EFFECTS OF ENDOTOXIN AND TNF/CACHECTIN IN MICE, R. Silverstein*, B.R. Turley*, C.A. Christoffersen*, D.C. Johnson*, and D.C. Morrison*, Univ. Kansas Med. Ctr., Kansas City, KS 66103

Hydrazine sulfate pretreatment protects normal mice against the lethal effects of endotoxin, (R. Silverstein, C.A. Christoffersen, and D.C. Morrison 1989 Infect. Immun.) and, in more recent studies, against the lethal effects of endotoxin and TNF-α in D-galactosamine-sensitized mice (J. Exp. Med., in press). With this latter model, the endotoxin LD_{50} is increased approximately four orders of magnitude. Protection is completely blocked, however, if D-galactosamine, a liver specific protein synthesis inhibitor, is present during the pretreatment period. This critical pretreatment period must be at least two hours for effective protection, during which time there is a transitory increase in circulating corticosterone, 41.8 ± 1.9 g/ml vs. 16.0 ± 0.1 g/ml ($P < .005$) at 30 min. The protection against endotoxin, both in normal and D-galactosamine sensitized mice, is abrogated by hypophysectomy, consistent with the possibility of a glucocorticoid-mediated mechanism, and our earlier finding of sustained hepatic PEPCK levels following LPS challenge. Hydrazine sulfate protection against injected TNF-α persists in hypophysectomized mice. Other differences between hydrazine protection against LPS vs. TNF-α challenge include the time of hydrazine sulfate pretreatment that is needed, and sensitivity to the presence of D-galactosamine during the pretreatment period. While the detailed mechanism(s) of the protection remain to be elucidated, it would appear that the dramatic protection afforded against endotoxin in D-galactosamine sensitized mice may involve a pituitary/adrenal axis with the liver a possible target of the endocrine involvement.(Supported by AI22948).

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IMPORTANT STEROID METABOLISM MODIFICATIONS DURING HUMAN AND EXPERIMENTAL SEPTIC SHOCK. N. Christeff, A. Carli (°), M.C. Auclair (°°), N. Thobie, C. Benassayag and E.A. Nunez. U.224, INSERM, Faculté Xavier Bichat, 75018 Paris - (°) Service de Réanimation Polyvalente, CHU Cochin, 75674 Paris - (°°) U.228, INSERM, 75006 Paris.

In men, during septic shock, the circulating concentrations of estrogens increase dramatically, while those of testosterone decrease. The increase in estrogen levels, particularly estrone, appears to be correlated with the outcome of septic shock : the patients who recovered had very high estrone concentrations on day one that decreased progessively during 48h ; the patients who died had as high estrone levels on day one, but they increased throughout the 48h.

These changes in sex hormone concentrations have been confirmed in experimental studies on male rats acutely treated with non-lethal doses of Escherichia Coli endotoxin (Endo). Endo administered in similar conditions to adrenalectomized or orchidectomized male rats did not induced these hormonal changes. Such results suggest that there are adrenal-testicular cooperation and activation of aromatase activity in the hormonal response to acute Endo injection. This adrenal-testicular cooperation has been confirmed by studying the hormonal response to Endo during development. The lack of an estrogen response to Endo injection in young rats is due to the absence of adrenal-testicular cooperation as a result of partial testicular immaturity.

Increase of aromatisation of androgens to estrogens has been confirmed by the work on the effect of an aromatase inhibitor on steroid variations induced by Endo. In male rats treated by aromatase inhibitor (4-hydroxyandrostenedione), the large increase in estrogen levels induced by Endo was not observed. The androgen levels remained very elevated.

We have also observed after treatment with adrenal hormones followed by Endo injection a decrease in DHEA sulfate and an increase in androstenedione.

The steroid modifications shown in these studies may form the basis for further therapeutic approaches in human septic shock.

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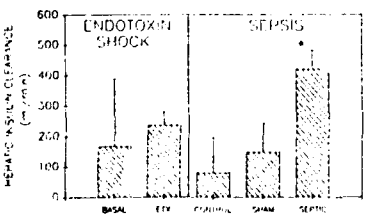
HEPATIC INSULIN CLEARANCE DURING SEPSIS OR ENDOTOXIN SHOCK. M.P. McLane*, G.C. Zenni*, W.R. Law, and R.M. Raymond. Depts. of Surgery and Physiology, Loyola University Medical Center, Maywood, Illinois, USA, 60153 and VA Hospital, Hines, IL, USA 60141.

Hyperinsulinemia has been observed during sepsis. The hyperinsulinemia may be due to increased secretion from the pancreas and/or decreased clearance of insulin by the liver. There have been reports of increased insulin secretion during sepsis/endotoxin shock but the ability of the liver to clear insulin during sepsis has yet to be established. This study was undertaken to investigate hepatic insulin clearance (HIC) during sepsis or endotoxin shock. For the sepsis study, mongrel dogs were assigned to 3 equal sized groups (control, sham, septic;n=7/group). Sepsis was induced by placement of a fecal-soaked sponge amid the intestines. HIC was studied on the seventh day of sepsis. Clearance Prep: Dogs were anesthetized with pentobarbital (30mg/kg), intubated and mechanically ventilated. Via a left-side laparotomy, electromagnetic flow probes were placed on the common hepatic artery and the superior mesenteric artery (SMA) to measure blood flows. By ligating all arterial inflows to the portal vein (PV) except the SMA, SMA flow was equivalent to PV flow. Catheters were placed in the PV, hepatic vein and femoral artery for blood sampling. HIC was calculated as the ratio of the rate of hepatic insulin uptake/pre-hepatic insulin concentration. Following stabilization HIC was determined. For the separate endotoxin (ETX) study, healthy mongrel dogs (n=4) underwent the above described Clearance Prep.



After stabilization, endotoxin (S.typhimurium, 1mg/kg, i.v.) was administered. Insulin clearance was determined pre-ETX and 60 min post-ETX administration. Endotoxin caused no changed in HIC (See left panel of Figure). HIC prior to exogenous insulin infusions during sepsis was significantly increased (sepsis 419.1 ± 61.2 ml/min vs control 80.1 ± 115.9 and sham 147.8 ± 95.2 ml/min, see right panel of Figure). Conclusions: Sepsis but not endotoxin shock increased in vivo hepatic insulin clearance.

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REVERSAL OF HEPATIC SUBSTRATE PREFERENCE DURING SEPSIS BY PHENYLEPHRINE BUT NOT GLUCAGON STIMULATION. C.N. Paidas*, M.M. Chin* and M.G. Clemens, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205 USA

The aim of this study was to determine relative hepatic substrate preference for nitrogenous (alanine, Ala) or non-nitrogenous (lactate, LAC) precursors in sepsis (S) and whether it is altered by hormone stimulation. Rats (250gm) were sham-operated or made septic by cecal ligation and single puncture (hyperdynamic at 24 hrs). Livers were perfused with Krebs buffer + RBCs using 1mM ^{14}C-Ala + 1,5,10mM unlabelled LAC (n=5 each group) without hormone (C) or with either phenylephrine (5μM, PE) or glucagon (10nM, GLC). Urea production (UP,mg/100g/h), glucose from Ala (^{14}C-GP, μmol/g/h) and CO_2 from Ala ($^{14}CO_2$) were determined. Results: mean±SEM. *p<0.01 vs Sham

	LAC	^{14}C-GP			$^{14}CO_2$			UP		
	mM	C	GLC	PE	C	GLC	PE	C	GLC	PE
Sham	1	8±.2	6±.2	12±.2	15±.3	18±.4	10±.3	.16±.01	.23±.01	.42±.01
	5	8±.1	5±.1	13±.3	19±.3	22±.8	9±.2	.17±.01	.24±.01	.43±.01
	10	8±.2	5±.1	14±.2	23±.3	26±.4	9±.2	.18±.01	.28±.01	.43±.01
Sepsis	1	4±.2*	2±.1*	9±.2*	9±.3*	17±.2*	5±.2*	.25±.01*	.34±.01*	.13±.01*
	5	3±.2*	1±.01*	12±.3*	23±.2*	32±.3*	2±.1*	.28±.01*	.42±.01*	.14±.01*
	10	4±.2*	.2±.01*	15±.3*	33±.4*	45±.8*	1±.1*	.33±.01*	.52±.01*	.13±.01*

Without hormones, GP by S livers was less than sham but LAC stimulated Ala oxidation and increased UP; GLC magnified this phenomenon. In contrast, PE stimulated GP from Ala but markedly inhibited both Ala oxidation and UP during S. These data demonstrate preferential oxidation of Ala during C and GLC stimulation in S livers. With PE, both sham and S livers utilize LAC as preferred fuel (confirmed by additional experiments with labelled LAC). Thus hepatic substrate preference is altered in S and appears sensitive to the second messenger system activated. Supported by NIH Grant GM 12580.

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SERIAL CHANGES IN PLASMA SUBSTRATES AND HORMONES SHORTLY AFTER ACCIDENTAL INJURY. R.N.Barton*, M.O.Doyle*†, R.A.Cocks*†, H.Chambers*† and A.White*§ (Spon.: R.A.Little). North Western Injury Research Centre and Departments of †Accident and Emergency Medicine and §Medicine (Clinical Biochemistry), Hope Hospital, Salford M6 8HD, U.K.

Single blood samples have given much valuable information on the concentrations of substrates and hormones in recently injured people (Stoner et al. 1979). However, the data are very variable and it is not known whether this largely reflects differences in response between patients or whether there are also rapid changes with time within patients. This variability is particularly striking for ACTH (Barton et al. 1987). To help answer this question, we have measured plasma substrate and hormone concentrations serially in 14 patients with accidental injuries, ISS 9-13. At least five blood samples were taken from each one at about 20-min intervals within the first 165 min after injury. Overall, plasma ACTH showed the same sort of variability as we observed previously. In half the patients, all with single fractures, ACTH fell throughout the study period. In the other half, it again varied, but in a less systematic way; in three of these it was high throughout. With a few possible exceptions, the changes in plasma cortisol were appropriate to those in ACTH. Of the metabolites studied, glucose showed no change during the study period, while lactate usually fell. Plasma free fatty acids (FFA) and β-hydroxybutyrate rose in most patients, the mean concentrations towards the end of the study period being about twice those towards the beginning. This was probably due to decreasing FFA re-esterification within adipose tissue, since there was only a slight rise in plasma glycerol. Although some of these changes may have been due to treatment, we advise caution in interpreting data from single blood samples after injury.

Stoner, H.B. et al. (1979) Clin. Sci. 56: 563-573
Barton, R.N. et al. (1987) J. Trauma 27: 384-392

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EFFECT OF PROXIMAL FEMUR FRACTURE ON CORTISOL KINETICS IN OLD PEOPLE. M.A. Horan*, R. Fisher*† and R.N. Barton*† (Spon.: R.A. Little). Dept. of Geriatric Med. and †N.W. Injury Research Centre, Hope Hospital, Salford, U.K.

Elderly people with proximal femur fracture have persistently raised plasma cortisol concentrations. At the European Shock Society meeting in 1990 (abstract 35) we reported that urinary free cortisol excretion in such patients was increased disproportionately to cortisol production rate, suggesting impaired clearance of cortisol from the circulation. We have now tested this directly by injecting [^{3}H]cortisol and following its disappearance from the circulation in 13 female patients (aged 71-92) about two weeks after hip fracture and in nine women (aged 67-83) known to be in excellent health. In agreement with previous studies in younger subjects, the disappearance curve for [^{3}H]cortisol approximated closely to a double exponential; the mean half-lives in the controls were about 5 and 70 min. In the trauma patients the slow half-life was greatly increased (on average to 120 min). The metabolic clearance rate (MCR) of cortisol was negatively correlated with this half-life in both groups of subjects; however, the difference in MCR between them was not quite significant. Indocyanine green clearance, measured as an index of hepatic blood flow, was correlated with MCR in the injured patients, and both variables were negatively correlated with age. In the controls the relationships were similar but the correlations were not significant. The mean plasma cortisol concentration during the three-hour period after injection was, as expected, higher in the patients than in the controls. In four of the control subjects, infusion

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of cortisol to raise its plasma concentration to the upper end of the range in the injured caused a rise of about 30% in MCR. Thus, had the patients been studied at the same cortisol concentration as the controls the apparent fall in MCR after injury would have been slightly greater. Nevertheless, this fall was still small and was probably due mainly to the greater age of the patients rather than to the injury per se.

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EFFECTS OF ETHANOL ON THE REDUCTION IN BAROREFLEX SENSITIVITY INDUCED BY SCIATIC NERVE STIMULATION. E. Kirkman, H.W. Marshall*, J.R. Banks*† and R.A. Little.
NWIRC and †Dept. of Physiol. Sci., University of Manchester, Manchester M13 9PT, U.K.

Peripheral musculo-skeletal injury produces a reduction in baroreflex sensitivity (1,2) due to an activation of nociceptive afferent fibres (2). Since accidental injury is often associated with ethanol intoxication, the aim of the current study was to determine the effects of ethanol on the changes in baroreflex sensitivity induced by electrical stimulation of the central cut end of the sciatic nerve (SCI), which activates afferent fibres including those relaying nociceptive information. All tests were conducted in held expiration induced by stimulating the superior laryngeal nerves. Six beagle dogs (10.0-21.7 kg) were anaesthetized with propofol (0.5 $mg.kg^{-1}$ bolus, then 0.3-0.8 $mg.kg^{-1}.min^{-1}$ iv). Baroreflex sensitivity was assessed using a bolus injection of phenylephrine (5.5-20.0 $ug.kg^{-1}$ iv; 3). In each of the 6 dogs SCI produced a significant ($p<0.05$, t test) reduction in the sensitivity of the baroreflex (7.01±1.70 $ms.mmHg^{-1}$ pre-SCI; 3.04±0.10 during SCI; group means ± s.e.m.). An intravenous infusion of ethanol producing plasma levels of 169.8±23.7 mg% had no consistent effect on baroreflex sensitivity. Furthermore, SCI still produced significant reductions in baroreflex sensitivity (8.39±0.32 pre-SCI; 1.51±0.07 during SCI); indeed, the effect was enhanced in 4 of the 6 dogs. These results indicate that the reduction in baroreflex sensitivity induced by SCI (and possibly injury) is preserved, and may be enhanced, in the presence of ethanol.

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2. Redfern, W.S. *et al.* (1984). Q.J. exp. Physiol. **69**, 763-779.
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REGULATION OF THE GENE EXPRESSION OF PREPROENKEPHALIN IN THE RAT BRAIN: INFLUENCE OF HEMORRHAGIC SHOCK. L. Fan* and T. K. McIntosh.
University of Connecticut Health Center, Farmington, CT 06032

We have previously reported that acute hemorrhagic shock is accompanied by an increase in preproenkephalin (ppENK) mRNA levels in the spinal cord of rats. The present study explores the influence of acute hemorrhagic shock on the expression of ppENK mRNA in specific regions of the rat brain. In male Sprague-Dawley rats (250-350g) femoral arterial and venous catheters were inserted under sodium pentobarbital anesthesia (50mg/kg). All animals were allowed to recover in their home cages. The following day, awake animals were bled to a constant blood pressure of 50 mmHg for one hour (n=10) and two hours (n=10) via a femoral arterial PE-50 cannulae. Control animals (n=15) were subjected to surgery but not bled. At designated time points, animals were sacrificed, brains removed and dissected into parietal cortex, hippocampus, diencephalon, brainstem, hypothalamus. Spinal cord tissue was also dissected. Total RNA was isolated from brain tissue using hot phenol/chloroform method. Preproenkephalin mRNA levels were quantified by Northern-blot hybridization of total RNA with a 32P-labeled cDNA probe and autoradiograms were scanned with a densitometer. The ppENK mRNA levels in parietal cortex and spinal cord were significantly increased after 1 hour of hemorrhagic shock when compared with control group ($P<0.05$). The ppENK mRNA concentrations in the diencephalon were significantly increased following two hours of hemorrhagic shock ($P<0.01$). In the brainstem, ppENK mRNA levels were significantly decreased after both 1 and 2 hours of hemorrhagic shock compared with control values ($P<0.01$). No significant changes were observed in the hippocampus or hypothalamus after 1 or 2 hours of hemorrhagic shock. These results indicate that during acute hemorrhage, the biosynthesis of enkephalin is altered in specific brain regions. It is possible that the decrease in brainstem ppENK mRNA may be a protective mechanism which reduces certain inhibitory effects of enkephalins on important brain cardiovascular regulatory centers.

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BIPHASIC REDISTRIBUTION OF ALPHA-ADRENERGIC RECEPTORS BETWEEN PLASMA MEMBRANE FRACTION AND INTRACELLULAR (LIGHT) VESICLES IN RAT LIVER DURING SEPSIS. Tsann-Long Hwang*, Ying-Tung Lau*, and Miin-Fu Chen*, Dept. of Surg. and Physiol., Chang Gung Med. Coll., Taiwan, ROC, and Chaoshu Tang*, and Maw-Shung Liu, Dept. of Pharmacol. and Physiol. Science, St. Louis Univ. Sch of Med., St. Louis, MO 63104, USA.

Changes in the distribution of alpha-adrenergic receptors in two subcellular fractions, the plasma membrane and the light vesicle, of rat liver were studied by photoaffinity labeling technique using [^{125}I]aryl azidoprazosin as a ligand during sepsis. Sepsis was induced by cecal ligation and puncture (CLP). The results on sodium dodecyl sulfate-polyacrylamide gel electrophoresis show that two major binding subunits with M_r of 77,000 and 68,000 and one minor binding component with M_r of 39,000 were labeled and visualized in both fractions. During early sepsis (9 hr post CLP), the total binding for three labeled peptides was increased by 26% ($p<0.01$) in plasma membranes but was decreased by 33% ($p<0.01$) in light vesicles. During late sepsis (18 hr post CLP), the total binding for three labeled peptides was decreased by 19% ($p<0.01$) in plasma membranes but was increased by 35% ($p<0.01$) in light vesicles. These data indicate that alpha-adrenergic receptors in rat liver were externalized from the intracellular vesicles to the plasma membrane fraction during early sepsis, but they were internalized from the plasma membranes to the intracellular sites during late sepsis. The [^{3}H]prazosin binding studies further confirms the externalization

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during early and the internalization during late sepsis of alpha-adrenergic receptors in rat liver. Since alpha-adrenergic mediation plays a role in the control of hepatic glucose metabolism by catecholamines, the present findings will contribute to the understanding of the pathophysiology of altered hepatic glucose homeostasis during sepsis. (Supported by grants from NIH (GM-31664 and HL-30080), NSC 79-0419-B182A-27 (ROC) and NMRP080 (Chang Gung Med. Coll.)).

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BIPHASIC CHANGES IN THE MUSCARINIC CHOLINERGIC RECEPTORS IN RAT HEART DURING SEPSIS. Ying-Tung Lau*, Dept. of Physiol., Chang Gung Med. Coll., and Tsann-Long Hwang*, Miin-Fu Chen*, and Chau-Hsiung Chang*, Dept. of Surg. and Cardiovasc. Surg., Chang Gung Memorial Hospital, Taiwan ROC, and Chaoshu Tang*, Xiang-Ying Chen*, and Maw-Shung Liu, Dept. of Pharmacol. and Physiol. Science, St. Louis Univ. Sch. of Med., St. Louis, MO 63104, USA.

Changes in the number of muscarinic cholinergic receptors in two subcellular fractions, the sarcolemma and the light vesicle, of rat heart were studied by affinity labeling technique using [^{3}H]propylbenzilylcholine mustard as a ligand during sepsis. Sepsis was induced by cecal ligation and puncture (CLP). The results on sodium dodecyl sulfate-polyacrylamide gel electrophoresis reveal that a single binding component with M_r of 80,000 was labeled in both membrane fractions. During early sepsis (9 hr after CLP), the binding of the 80,000 M_r peptide was increased by 100% ($p<0.01$) in sarcolemmal fraction but it remained unchanged in light vesicles. During late sepsis (18 hr after CLP), the binding of the 80,000 M_r peptide was decreased by 20% ($p<0.01$) but it remained unaltered in light vesicles. These data indicate that muscarinic cholinergic receptors in rat heart sarcolemma was hypersensitized during early but desensitized during late sepsis with no apparant translocation to the intracellular sites. The [^{3}H]quinuclidinyl benzilate binding studies of membrane preparations further confirms the [^{3}H]propylbenzilylcholine mustard results. Since muscarinic cholinergic receptors mediate parasympathetic modulation of myocardial contractility, changes in the number of muscarinic receptors in cardiac sarcolemma may have a pathophysiological significance in contributing to the development of myocardial dysfunction during sepsis. (Supported by grants from NIH (HL-30080 and GM-31664) and NMRP080 (Chang Gung Med. Coll.)).

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EXTERNALIZATION AND INTERNALIZATION OF BETA-ADRENERGIC RECEPTORS IN RAT HEART DURING SEPSIS. Maw-Shung Liu and Chaoshu Tang*, Dept. of Pharmacol. and Physiol. Science, St. Louis Univ. Sch. of Med., St. Louis, MO. 63104.

Changes in the distribution of beta-adrenergic receptors (ßAR) in two subcellular fractions, the sarcolemma and the light vesicle, of rat heart were studied by using ^{3}H-dihydroalprenolol as a ligand during sepsis. Sepsis was induced by cecal ligation and puncture (CLP). The results show that during early sepsis (9 hr post CLP), the Bmax was increased by 34 % ($p<0.01$) in sarcolemmal fraction but was decreased by 24 % ($p<0.01$) in light vesicles. During late sepsis (18 hr post CLP), the Bmax was decreased by 38 % ($p<0.01$) in sarcolemma but was increased by 30 % in light vesicles. Addition of Gpp(NH)p to the sarcolemmal membranes shifted the agonist displacement curves to the right in the control and early sepsis groups but failed to affect that in the late sepsis group. Addition of Gpp(NH)p to the light vesicles had no effect on the agonist displacement curves in all three groups (control, early and late sepsis). These data indicate that ßAR was externalized from the intracellular sites to the sarcolemmal membranes during early sepsis, but was internalized from sarcolemma to light vesicles during late sepsis. Photoaffinity labelling of membrane preparations using ^{125}I-iodocyanopindolol diazirine confirms the externalization of ßAR during the early and the internalization during the late sepsis. Since myocardial contractility is controlled by catecholamines through ßAR mediation, a biphasic redistribution between the sarcolemma and the light vesicles may contribute to the development of myocardial dysfunction during sepsis. (Supported by NIH grants HL-30080 and GM-31664).

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NON-BARORECEPTOR MECHANISMS MEDIATE THE SYMPATHETIC RESPONSE TO ENDOTOXIN (ET) IN CONSCIOUS RATS. S.B. Jones, R.D. Wurster, M. Qi and Z.Z. Zhou. Loyola Univ. Medical Center, Maywood, IL 60153.

Hypotension that follows endotoxin treatment may mediate sympathetic activation associated with ET through peripheral baroreceptors. To test this hypothesis, male rats (350 g) underwent sinoaortic (SAD) or sham (SHAM) denervation, 2-4 week recovery followed by intravenous ET experiments. Prior to the protocol (24 hrs) rats were instrumented with femoral arterial and venous cannulae, and bipolar, gold electrodes on the renal branch of the splanchnic nerve (leads exteriorized back of the neck). In fasted, conscious rats, renal sympathetic nerve activity (RNA) and arterial plasma catecholamines were determined at 0, 30 and 60 min post ET (5.0 mg/kg S. enteritidis). RNA was amplified ($5x10^4$-10^5 fold), filtered (35-2000 Hz), full wave rectified and integrated. Changes in nerve activity (minus background) are expressed as a percentage of control values for each animal. Plasma norepinephrine (NE) and epinephrine (E) were determined by radioenzymatic analysis (duplicate 50 µL plasma samples). SAD was confirmed with plasma NE and E, RNA and heart rate responses to hypotension (data not shown). Results:

Time Post ET - (minutes)		0	30	60
RNA(% control)	SAD (7)	100	198±30	211±38
	SHAM (7)	100	213±34	233±35
NE(pg/mL)	SAD (4)	485±129	3802±575	3089±415
	SHAM (5)	215±60	3354±605	2937±764
E(pg/mL)	SAD (4)	471±163	14946±2260	7468±927
	SHAM (5)	140±23	9583±2060	7108±1625

No differences found with group t-test, SAD vs SHAM at each time. $\bar{X}$±SEM, (N).
Results demonstrate that elimination of baroreceptor function does not impair sympathetic response to ET treatment and in some cases responses were greater in SAD rats. It is concluded that mechanisms other than baroreceptor are likely to mediate the major component of the sympathetic response to endotoxin. (Supported by the Bane Foundation).

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ENDOTOXIN-AGE-DEPENDENT ESTROGEN RESPONSE IN MALE RATS.

N. Christeff, M.C. Auclair(*), N. Thobie, C. Benassayag and E.A. Nunez. U.224, INSERM, Faculté Xavier Bichat, 75018 Paris - (*) U.228, INSERM, 15, rue de l'Ecole de Médecine, 75006 Paris.

The influence of acute endotoxin (Endo) administration on adrenal and testicular serum hormones, corticosterone (B), progesterone (P4) 17αOH progesterone (17αOH P4), androstenedione (Δ4), testosterone (T), estrone (E1) and estradiol (E2) was studied in male rats aged 8,12 and 15 weeks. The concentrations of circulating steroid hormones in male rats (controls) vary with age showing that adrenal glands mature before the testes. The steroid response to Endo is age-dependent. B, P4, 17α OH P4 was increased and T decreased in all animals. But, there was a very significant increase in estrogens (E1, and E2) and a decrease in Δ4 only in male rats aged 12 weeks and over.

The most significant age-dependent change in hormone levels in response to Endo appear to be the increase in E2 at 12 weeks of age or older. As suggested by our previous work, the lack of an estrogen response to Endo injection which occurs in young rats is due to the absence of adrenal-testicular interaction as a result of partial testicular immaturity.

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THE KAPPA OPIOID RECEPTOR ANTAGONIST NALMEFENE IMPROVES OUTCOME IN A MODEL OF FIXED VOLUME HEMORRHAGIC SHOCK IN THE RAT. Tracy K. McIntosh, Sharon B. Samuels and Neil S. Yeston. Surgical Research Center, University of Connecticut Health Center, Farmington, CT 06032.

The cardiovascular sequelae of hemorrhagic shock appears to be affected by endogenous opioid peptides acting at central and peripheral opioid receptors. Novel compounds selective for specific opioid receptor subtypes have recently been synthesized, allowing for the elucidation for specific receptor mechanisms underlying cardiovascular dysfunction. The present study examined the efficacy of the novel opiate antagonist nalmefene, which has enhanced activity at the dynorphin/kappa receptor, in improving cardiovascular dysfunction and mortality in severe fixed-volume hemorrhagic shock in rats. Male Sprague-Dawley rats (n=20) underwent left femoral artery and right jugular vein cannulation under pentobarbital anesthesia. A thermistor was inserted into the aortic arch via the left carotid artery for cardiac output/stroke volume (CO/SV) monitoring. The following day, the thermistor was connected to a Cardiomax-II CO computer and the jugular catheter to a microinjector. Awake animals were bled approx 50% of their blood volume (9.5 cc/300 g b.w.). This model typically results in a 63% mortality in control animals in less than 5 hours. Fifteen minutes after the completion of hemorrhage, animals randomly received an intravenous injection of nalmefene (0.1 mg/kg, n=7) followed by a constant infusion (0.1 mg/kg/hr) for 8 h, nalmefene (1.0 mg/kg, n=6) followed by a constant infusion (1.0 mg/kg/hr) for 8 h or saline (equal volume, n=7). By 20 minutes post-treatment, animals treated with nalmefene (0.1 mg/kg dose) showed a significant improvement in mean arterial blood pressure (mean increase = 16mmHg; p,0.05) and in cardiac output (mean increase = 12 ml/min; p,0.05) when compared to saline-treated controls. Animals treated with the higher dose of nalmefene (1.0 mg/kg plus infusion) showed no improvement in cardiovascular function during shock. No significant differences were observed in mortality between the groups. These results suggest that the kappa-opiate receptor antagonists such as nalmefene may have beneficial effects in the treatment of hemorrhagic shock.

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EFFECT OF THE GLUCOCORTICOID ANTAGONIST, RU 38486, ON SURVIVAL AND TNF PRODUCTION DURING SEPTIC AND ENDOTOXIN SHOCK. G. Lázár Jr.*, G. Lázár *, E. Duda *, M.K. Agarwal * (Spon: S. Nagy)
Deparment of Surgery, Institute of Pathophysiology, Albert Szent-Györgyi Med.Univ., Biol. Res. Ctr of Acad. Sci. Hung. H-6720 Szeged Hungary, and Hormonologie, Univ. Pierre et Marie Curie, Paris, France.

Glucocorticoid hormons, and bacterial endotoxins contribute to the eventual outcome of a wide array of pathological syndromes in humans, but their precise role remains unclear. Tumour necrosis factor (TNF) is released in response to bacterial lipopolisacharide (LPS), and it has been implicated as a principal mediator of endotoxin and septic shock. We have investigated the effect of the potent antiglucocorticoid, RU 38486, on the survival rate of mice, undergoing septic peritonitis following cecal ligation and puncture, and after administration of E.coli 026:6B LPS. A single intravenous dose of 1 mg of RU 38486, concurrent with puncture, lowered the survival to 15% from the control level of 71%. Similarly, the antihormone decreased the survival to 35% from 90% in the group of mice made tolerant to LPS. RU 38486 also abolished the tolerance to LPS, which is possibly responsible for the resistance to septic peritonitis. The survival of mice decreased to 70%, 15%, and 5%, if a single dose of 0.25, 0.5, or 1 mg RU 38486, respectively, was administered,compared to the 90% of survival rate in control animals which had been treated with 0.5 mg LPS. The sensitizing effect of the antihormone was not attributed to altered retention of 51Cr labelled LPS in liver, lung, spleen, or blood. We have found the RU 38486 significantly increased the level of TNF in blood, liver and spleen after endotoxin challenge. These results outline the significance of TNF in the pathogenesis of septic and endotoxin shock. The physiological action of RU 38486 is believed to proceed via the glucocortocoid receptor (GCR) in vivo, and endotoxins alter GCR function in a number of organs. Therefore our findings seem to prove, that a glucocorticoid-dependent mechanisms have an important role in forming the resistance to septic and endotoxin shock.

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S10: Models of Organ Failure and Sepsis

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ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS-INDUCED SHOCK IN THE NONHUMAN PRIMATE: ASSESSMENT OF MULTIPLE ORGAN FAILURE. L.B. Hinshaw and T.E. Emerson, Jr., Oklahoma Medical Research Foundation, Oklahoma City, OK 73104 and Cutter Biological, Miles Inc., Berkeley, CA 94701 (USA).

This study was undertaken to determine the degree of injury of various organ systems in a nonhuman primate subjected to shock by E. coli or S. aureus organisms. Previously reported animal shock models have been studied without regard to correlations between multiple organ failure and survival time: death occurred too early for development of tissue damage. Studies concluded after a period of less than one day lack relevance to the typical human patient. The foregoing experiments were conducted on seven anesthetized juvenile or young adult baboons, Papio c. cynocephalus, given intravenous doses of E. coli or S. aureus, 2-4 x 10^{10} CFU/kg in a 2 hr period. Antibiotic was administered after the infusion of organisms and subsequently for three days. Animals were monitored for 10 hours and observed continuously for 30 hours, daily for 7 days or until death. Baboons were supported with a therapeutic regimen for 10 hours which extended life but did not prevent death. Survival times were: E. coli, 2.4 to 6.9 days (mean, 4.8); S. aureus, 3.8 to 4.7 days (mean 4.4). Gross and light microscopical examinations were conducted to assess the degree and character of multiple organ failure. Organ pathology was rated from +1 (mild) to +4 (severe). Results were as follows: E. coli (n=4):lungs +3 (n=2); adrenals +4 (n=4); kidneys +3 (n=3); liver +2 (n=2); spleen +2 (n=2); heart +2 (n=2); S. aureus (n=3): lungs +3 (n=3); adrenals +2 (n=3); kidneys +3 (n=3); liver +2 (n=3); spleen +2 (n=2). Congestion, hemorrhage, fibrin deposition and tissue necrosis were the hallmarks of each form of shock. In summary, findings in the semi-chronic baboon model of severe sepsis provide evidence for the association of significant multiple organ damage and the prolongation of shock and create opportunities for new therapies to be applied in the later phase of shock.

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STAGING AND MOLECULAR MECHANISMS OF THE BABOON RESPONSE TO E.COLI: DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS. Fletcher B. Taylor Jr.,
Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

First, the baboon response to lethal concentrations of E.coli will be discussed in detail. The response is divided into four stages: Inflammatory, coagulopathic, cell injury and cell degeneration. The role of TNF, PMN leukocytes and their interaction in the inflammatory (stage I) response will be discussed. Then the role of the protein C and protein S anticoagulant systems and the generation of tissue factor by vascular endothelium in the coagulopathic (stage II) response will be discussed. Of particular interest is the influence of the acute phase protein, C4bBP on the response of the anticoagulant system and amplification of the inflammatory response. Finally, the role of oxygen free radicals in the cell injury (stage III/IV) response will be discussed with emphasis on the endothelium as a source of free radicals. Second, the baboon response to sublethal concentrations of E.coli will be discussed with emphasis on 1) assays of plasma factors which distinguish between a response which is controlled (sublethal) versus one which is uncontrolled (lethal), and 2) the diagnostic and prognostic implications of these assays. Third, variants of the baboon response to E.coli will be discussed with emphasis on 1) capillary leak syndrome and the role of TNF, 2) the hemolytic uremic syndrome and the role of C4bBP.

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A SUBCHRONIC MODEL OF LIVE BACTERIA SEPSIS IN BABOONS. G. Schlag, J. Davies*, H. Redl, C.J.J. van Vuuren* and D. Robertson+, Ludwig Boltzmann Institute Exp. Clin. Traumatol., Vienna, Austria; Roodeplaat Research Lab., Pretoria, South Africa. + In memoriam of D. Robinson.

We have set up a sepsis model with live E. coli in baboons with a mortality rate of about 50 - 75 % within 72 hours under additional intensive care conditions (fluid administration, supported ventilation in certain intervals). Eight adult male baboons (21 - 25 kg) were anesthetized with pentobarbital sodium (intubated with spontaneous breathing) and instrumentated for hemodynamic measurements according to our previous procedures (G. Schlag et al. Circ.Shock 1991, in press). The animals were infused with E. coli (5 x 10^8 - 1 x 10^9 CFU/kg body weight) over 2 hours and observed for further two hours. After termination of the acute stage (4 hours after start of E. coli infusion), the animals were extubated and the Swan Ganz catheter was removed, leaving the introducer and the arterial catheter (brachial artery). The skin incisions were closed by sutures. For each measurement during the awake subchronic stage, the animals were sedated with ketamin hydrochloride (Ketalar, 3 - 5 mg/kg body weight) and intubated (supported ventilation); a subcutaneous pouch was opened under sterile conditions and measurements were made. Fluid was infused according to PWP until baseline values were restored. The wounds were closed again after each procedure (10h, 24h, 48h and 72h).

Results: Survival/non-survival 72h 2/6 (75% mortality)

Postmortem: development of multiorgan failure (lung, liver, adrenals, blood, brain, heart) dependent on the length of survival time.

Conclusion: Chronic instrumentation in the septic model is necessary to provide adequate fluid support and to create conditions similar to the human critical care setting as a precondition to test new therapeutic regimens.

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A CHRONIC OVINE MODEL OF HYPERDYNAMIC SEPSIS. W.J. Sibbald, Program in Critical Care, University of Western Ontario, London, Ontario, Canada.

Sepsis is considered a "host response" which is associated with evidence of diffuse inflammation, and usually complicates a well-defined focus of infection. The circulatory sequelae of the sepsis syndrome may be clinically defined as a spectrum, ranging from a hyperdynamic central circulatory state through to a hypodynamic and hypotensive circulatory state. It is intuitive that opportunities for modifying organ injury, thereby outcome, are improved when this syndrome is treated in its earliest stages of presentation. Therefore, we sought to develop an ovine model of hyperynamic sepsis which would be more representative of the early, than the later clinical stages of this syndrome.

We employed cecal ligation and perforation in sheep to induce an intraperitoneal inflammatory focus. Over the subsequent 24 to 48 hours, evidence of pulmonary dysfunction develops, and morphologic characteristics are similar to early ARDS. Widespread tissue injury is also evident at the extrapulmonary level, and is accompanied by biochemical evidence for organ dysfunction. For example, hepatic injury is accompanied by a modest elevation in bilirubin and depressed serum albumin. Importantly, the underlying injury in both pulmonary and extrapulmonary organ systems involves a microcirculatory lesion resulting in increased endothelial permeability to water and solutes of higher molecular weight, a process which results in excess organ edema. At the microcirculatory level within the extra- pulmonary organs, morphologic evidence of endothelial dysfunction may also explain patchy cell death noted in various organs.

The evidence for widespread tissue injury accompanying the host response to sepsis in this model is associated with significant abnormalities in the circulatory control of tissue O_2 flux. Even in the earliest stages of this model's response to cecal ligation and perforation, a maldistribtution in blood flows between organs depresses the flow reserve to the *splanchnic circulations*. For example, when cardiac output is depressed, the anticipated redistribution in blood flow from the gut to "vital" organs is substantially depressed. This finding is most likely explained by depressed flow and/or extraction reserve in the splanchnic circulations.

In summary, this ovine model of the host response to a focus of intraperitoneal infection demonstrates that sepsis is characterized by a defect in microvascular integrity in both pulmonary and extrapulmonary circulations. Concurrently, evidence fo dysregulation in the metabolic control of tissue O_2 delivery is apparent at all of the microcirculatory, regional and central levels of the circulation, and diffuse tissue injury results.

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THE EFFECTS OF HYPOVOLEMIA AND A SEPTIC FOCUS ON ORGAN FUNCTION. G.I.J.M.Beerthuizen, D.N.Herndon, B.Curry, L.Traber, D.L.Traber.
UTMB & Shriners Burns Institute. Galveston, Texas. University of Nijmegen, the Netherlands.

Tissue hypoxia could influence the outcome in sepsis and subsequent development of multiple organ failure. The effects of a large subcutaneous abscess and tissue hypoxia on liver, kidney, heart and lung function were studied. METHODS: In instrumented sheep (n=15) a large subcutaneous pocket of gauze was implanted and injected with staph.aureus ($6x10^{10}$ cfu). In one group (n=7) a period of 4 hours of hypovolemia was added. After 48 hrs histologic examination was performed. Data were analyzed by ANOVA-test. RESULTS: During the period of hypovolemia cardiac output (3.3+0.4 vs 6.3+0.33), mean arterial blood pressure (70+4 vs 94+6), art.PCO_2 (32+2 vs 39+2). DO_2 (347+36 vs 709+39) and skeletal muscle PO_2 (0.3+0.1 vs 2.7+0.9) decreased compared to the infection group alone ($p<0.05$). Arterio-venous oxygen difference (6.8+0.6 vs 3.9+0.1) and EO_2 (0.64+0.03 vs 0.34+0.01) increased during the period of hypovolemia ($p<0.05$). In both groups lung damage occurred. In the infection group without hypovolemia the lungs of all 8 animals showed marked congestion and edema while in the group with hypovolemia severe lung damage was observed in 3 out of 7 animals. No liver damage occurred in the infection group without hypovolemia while the liver of the animals subjected to hypovolemia showed focal necrosis ($p<0.05$). CONCLUSIONS: A large subcutaneous abscess causes lung damage more pronounced in the group without hypovolemia. No liver damage was found in the group with a septic focus alone, while in the group with hypovolemia focal liver cell necrosis was found. Hypovolemia resulting in tissue hypoxia in the presence of a septic focus promotes the development of multiple organ failure.

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MODELS OF ENDOTOXEMIA AND ACUTE LUNG INJURY. Daniel L. Traber
Univ. Tex. Med. Br. and Shriners Burns Inst. Galveston, Tx 77550-2788

We have utilized the sheep in studies of endotoxemia and inhalation injury for over a decade. These animals are large and docile, making it easy to study them in the unanesthetized state and for the collection of large blood samples. Their cardiopulmonary anatomy and function are similar to man. The administration of endotoxin in small quantities will induce pulmonary edema and an elevation in cardiac output similar to that seen in septic patients. Sheep and goats have lung lymphatics that can be cannulated for collection of lymph and the measurement of putative mediators of lung injury. Other lymphatics such as the tracheal, prefemoral, and popliteal vessels have also been studied in the unanesthetized state. The animals have large bronchial arteries enabling the study of the systemic blood flow of the lung. We have found it relatively easy to measure blood flow in mesenteric, celiac, renal, and femoral arteries. Ovine materials cross react with many monoclonal antibodies and RIA kits which are available. These include β-endorphin, angiotensin, and vasopressin. We have found it quite easy to use these animals for studies of myocardial contractility. We have placed piezo electric crystals on their hearts and with dimension analysis have been able to report cardiac volumes. It is also easy to obtain interventricular

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pressures and to calculate several indexes of contractility. For studies of acute lung injury, we found that the animals can adapt to positive pressure ventilation and to the presence of a tracheostomy tube. The animals are readily available and inexpensive ($100~$200).

CARDIOVASCULAR DYSFUNCTION IN SEPTIC SHOCK. J. E. Parrillo; Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, USA. **168**

Although sepsis usually produces a low systemic vascular resistance and elevated cardiac output, depression of myocardial function has been demonstrated in humans with septic shock. This cardiac dysfunction is characterized by a decreased left and right ventricular ejection fraction and a reduced response to fluid administration. Frequently, the ventricles are dilated and, in survivors, these abnormalities are reversible. Endotoxin administration to humans simulates the qualitative cardiovascular abnormalities of sepsis and confirms that myocardial depression is typical of this disease. Using a canine model of septic shock that is very similar to human sepsis, myocardial depression (assessed by load independent measures of ventricular function) was confirmed using bacteria, endotoxin, or tumor necrosis factor (TNF). Using an in vitro beating myocardial cell assay, both septic shock sera and tumor necrosis factor have been shown to produce a decrease in myocardial cell shortening. Pharmacologic or immunologic antagonism of the mediators producing myocardial depression may prove to enhance survival in septic shock.

EVALUATION OF CHEMICAL MEDIATORS IN A NEW ENDOTOXIN SHOCK MODEL BASED ON PLASMA ENDOTOXIN LEVELS USING THE ENDOSPECY TEST. T. Kubota, M. Yoshizawa and K. Ohtake. Jichi Medical School, Tochigi, Japan. **169**

The Endospecy test (ES test) provides accurate and qualitative measurement of plasma endotoxin (ET) levels in humans and animals. To produce a suitable animal experimental model similar to clinical septic shock, we assessed the kinetics of IV administration of ET using large doses (0.2-1.0 mg/kg) and low doses (0.02-0.002 mg/kg). At the same time we observed the correlation between hemodynamic and heamatological changes, plus the changes in humoral parameters.
Methods: Endotoxin shock was induced in mongrel dogs by single IV injection of ET either in a high dose or in a low dose. Blood samples were drawn serially for 6 hrs for the determination of plasma ET levels using the ES test. Hemodynamic parameters derived from a Swan-Ganz catheter and heamatological parameters (WBC, Plt counts) were observed simultaneously. Changes in humoral parameters (CH_{50}, prostaglandins, leukotriens and TNF) were examined for 6 hrs after ET was given.
Results: The peak plasma ET level was reached immediately after ET administration in all dogs. In the large dose group, ET levels remained remarkably high and out of range throughout the study. In the small dose group, ET levels gradually decreased and reached clinical septic shock levels. Leukocyte and platelet counts decreased immediately after ET injection in all groups. In the small dose group, TNF levels which showed a rapid increase at 60 min after ET injection, did not correlated to leukocyte and platelet decreases. However, arterial blood pressure decreased in accordance with TNF increase. A time difference was also observed on the initial changes between CH_{50}, prostaglandins and TNF.
Conclusion: The new endotoxin shock model seems to be a suitable, reliable method for evaluation of the pathophysiology of endotoxin shock, especially related to humoral mediator changes.

A MODEL OF SEPSIS AND SEPTIC SHOCK IN RODENTS. I.H. Chaudry, Departments of Surgery and Physiology, Michigan State University, East Lansing, MI 48824, USA **170**

Since sepsis continues to be the major cause of multiple organ failure and death in most intensive care units, it is essential to understand the relationship between sepsis and remote organ failure. Controlled studies in septic patients, however, are difficult to perform due to the diversity of diseases, organisms and other variables. Thus, it is important to develop reproducible animal models that simulate the pathophysiology of sepsis and septic shock in patients. There are numerous advantages in using rats and mice for such studies, since one can obtain genetically identical rodents of the same age, sex and specific pathogen free, thus minimizing biologic variables. Moreover, rodents are inexpensive and are widely available, allowing the use of large numbers of animals for statistical evaluation of results. Although various rodent models of sepsis have been proposed, such as slow or bolus endotoxin infusion, implantation of the rat's fecal material IP or subcutaneously, IP inoculum of bacteria (e.g. E.coli), we have developed the model of cecal ligation and puncture (CLP) to study the pathophysiology of sepsis and septic shock. CLP is an easy procedure in rats as well as mice, and blood cultures of such animals are positive for

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numerous enteric organisms within an hour following CLP. The animals progress from the early, hyperdynamic, high output sepsis to the hypodynamic, low output circulatory state during the late stages of sepsis. Thus, this model permits one to perform studies in the hyperdynamic as well as hypodynamic circulatory state. Using this model, various alterations in metabolism, immune responses and organ functions have been described with the progression of sepsis. This model also permits the slow induction of sepsis by decreasing the needle size for puncturing the cecum. In addition, excision of the ligated and punctured cecum to remove the septic focus is possible and thus, one can study the potential effects of various therapeutic modalities during either the high or low cardiac output septic state.

171 STIMULATION OF SUPEROXIDE ANION PRODUCTION AND IN VIVO GLUCOSE UTILIZATION BY HEPATIC PHAGOCYTES FOLLOWING IN VIVO ENDOTOXIN, LATEX PHAGOCYTOSIS, OR TNF CHALLENGE.

J.J. Spitzer, Z. Spolarics, *K. Meszaros, *A.P. Bautista, *A. Schuler, and C.H. Lang. Louisiana State Medical Center, New Orleans, LA 70112.

Our earlier investigations indicated that an LD_{15} E. coli endotoxin (LPS) challenge will increase gluconeogenesis and glucose utilization. The liver plays an important role in both effects. The aims of these investigations were to elucidate the contributions of the hepatic nonparenchymal cells to the LPS-induced increase in glucose utilization and to determine whether LPS will also prime the liver to produce superoxide anions. The effects of LPS were compared to those obtained after an in vivo phagocytic stimulus or TNF infusion. The rate of in vivo glucose utilization in the different cell types was measured by combining cell fractionation methods with the in vivo 2-deoxyglucose tracer technique. Generation of superoxide anions was determined by the reduction of ferricytochrome C in the presence or absence of PMA or opsonized zymosan, with or without superoxide dismutase (SOD). Glucose uptake by the Kupffer cells was increased markedly after all three challenges. Kupffer cells and sequestered neutrophils accounted for the major portion of increased hepatic glucose uptake. Isolated Kupffer cells and hepatic neutrophils released large quantities of superoxide anions following stimulation with PMA or zymosan. After in vivo LPS, latex or TNF, the perfused liver also released significant quantities of superoxide anions, which could be abolished by SOD, and markedly stimulated by PMA. We postulate that the increased glucose utilization is the metabolic support for the activated functional state of these cells and that the enhanced ability to produce toxic oxygen metabolites subserves the immune defense function of the cells. However, stimuli leading to the excessive production of toxic oxygen metabolites may also create a potential hazard, and result in liver injury and possibly hepatic failure (Supported by GM 32654).

172 REGIONAL BLOOD FLOW AFTER INTESTINAL ISCHEMIA-REPERFUSION INJURY

Richard Turnage*, Eddie Abdalla*, Todd Gerkin*, Michelle Imlay*, Kim Gallagher*, Karen Guice, Keith Oldham
Sections of General Surgery and Pediatric Surgery, Department of Surgery, University of Michigan Medical School, Ann Arbor, MI 48109

Reperfusion of ischemic intestine is associated with distant organ injury of the lung, liver, and kidney. Despite systemic hemodynamic stability, regional blood flow alterations may be involved in the injury process. The purpose of this study was to evaluate regional blood flow changes following intestinal ischemia-reperfusion. After insertion of carotid and femoral cannulae, male Sprague-Dawley rats underwent microvascular clip occlusion of the superior mesenteric artery (SMA) for 120 minutes. By removing the SMA clip, reperfusion was allowed for 0, 1, or 60 minutes. Immediately prior to sacrifice, the aortic root (or left ventricle) was injected with ^{141}Ce and ^{105}Ru labelled microspheres (15 μm). Tissue samples of distal small intestine, liver, pancreas, kidney, and skeletal muscle were obtained and analyzed to determine regional blood flow. Sham operated animals served as controls. Results:

	TISSUE BLOOD FLOW (ml/min/gm)				
GROUP	LIVER	DIST. INTESTINE	PANCREAS	KIDNEY	MUSCLE
0/180	0.38 ± 0.07	0.82 ± 0.06	0.50 ± 0.13	3.6 ± 0.55	0.08 ± 0.02
120/0	0.88 ± 0.42	0.25 ± 0.15*	0.60 ± 0.13	2.70 ± 0.93	0.29 ± 0.11
120/1	0.08 ± 0.06*	1.00 ± 0.34	0.29 ± 0.08	0.78 ± 0.37*	0.04 ± 0.01
120/60	0.07 ± 0.05*	0.22 ± 0.06*	0.11 ± 0.02*	0.52 ± 0.23*	0.05 ± 0.02

(*$p < 0.05$ versus sham, ANOVA; groups designated as minutes of ischemia/minutes of reperfusion)

Hepatic arterial and renal blood flows were reduced to 21% and 22%, respectively, of control levels immediately (1 min) after reperfusion despite a hyperemic response in the intestine and overall hemodynamic stability. This suggests that humoral or neurogenic mechanisms mediate these changes in blood flow. Blood flow in the 120/60 group was reduced in all organs and remained so in spite of crystalloid resuscitation (data not shown). We conclude that significant regional blood flow alterations, independent of volume status, occur with intestinal ischemia-reperfusion and that these alterations may make important contributions to distant organ injury.

173 REPERFUSION OF THE TRANSPLANTED LIVER LEAD TO ACTIVATION OF COMPLEMENT AND LEUKOCYTES.

H. Tomasdottir*, B-Å. Henriksson*, A. Bengtsson*, B. Norder*, M. Tylman*, (Spon: H. Haljamäe). Department of Anesthesiology & Intensive Care, Sahlgrens Hospital, 41345 Göteborg, Sweden.

The aim with the present study was to evaluate if reperfusion of the transplanted liver lead to activation of complement and of leukocytes.

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Complement activation (C5a*desArginine* and terminal complement complexes, TCC) and leukocyte activation (PMN elastase and neopterin) were determined preoperatively, 1 minute before start of reperfusion, 1, 15 and 60 minutes after start of reperfusion and 24 hours after start of operation in eight patients undergoing liver transplantation. Plasma concentrations of C5a*desArginine*, TCC, PMN elastase and neopterin were within the normal range preoperatively and 1 minute before start of reperfusion. One minute after start of reperfusion plasma TCC were elvated ($p<0.05$). 15 minutes later C5a*desArginine* were increased ($p<0.05$). One hour after start of reperfusion C5a*desArginine* and TCC as well as PMN elastase and neopterin were elevated compared to the concentrations found before start of reperfusion ($p<0.05$). These results indicate that the reperfusion lead to activation of complement and of leukocytes. The initiation of the complement cascade seems to appear earlier than the activation of the leukocytes. These effects may be one etiology behind complications like organ dysfunction, hypotension and circulatory insufficiency often seen in patients undergoing liver transplantation.

FINDING THE APPROPRIATE ANIMAL SEPSIS MODEL - A DECISION TREE APPROACH

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A. Lechleuthner*, S. Saad*, D. Rixen*, A. Braschoß*, E. Neugebauer

II. Dept. of Surgery and Biochem. and Exp. Divis., University of Cologne, Klinikum Merheim, Ostmerheimerstr. 200, 5000 Köln 91, FRG

Septic shock is still the most common cause of death in surgical ICUs. Currently a lot of clinical and experimental research is in progress to study this complex syndrome. For a specified question it is necessary to find out an appropriate animal model which optimally combines institutional facilities and the endpoint of interest. A decision tree was developed for structuring the selection process. Starting with a specific question or hypothesis one has to decide between a basic research direction (I) and a clinical research direction (II). In either category a second decision node asks for the endpoint of interest. Four categories are possible (1) mortality, (2) pathophysiological parameters, (3) pathobiochemical parameters, (4) others. For each direction and endpoint three selected model-designs (with subcategories) according to the orign of sepsis are offered: (a) endotoxicosis/bacteremia, (b) ischemia/peritonitis, (c) trauma/burn. For every model-design a structured data base with detailed descriptions and references is available for selection. The final step is to compare the model requirements with the institutional facilities by using a checklist. This decision tree approach is considered most useful in saving animal life, money and time.

IN VIVO ASSESSMENT OF REGIONAL MICROVASCULAR ALBUMIN LEAKAGE DURING *E.COLI* SEPTIC SHOCK IN THE BABOON MODEL.

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I.C. Dormehl, N. Hugo, J.P. Pretorius.
Faculty of Medicine University of Pretoria, P O Box 2034, Pretoria 0001, South Africa

Changes in regional microvascular albumin flux during septic shock were studied non-invasively by scintigraphy in the baboon model. Use was made of an i.v. injection of ^{99m}Tc-labelled baboon serum albumin. Count ratios of lung to cardiac, liver to cardiac and abdominal to cardiac regions were measured two-hourly for six hours in control and septic shock baboons, and compared. Increased ratios obtained during shock pointed to an increase in extravascular albumin. Linear regression lines fitted to these count ratios provided regional albumin leak indices. These indices (Table 1) demonstrated statistically significant increases ($P<0.05$) during septic shock for the abdominal region during the six-hour study, and for all regions, but especially the abdomen, when data were calculated over four hours. Increasing ratios and leak indices correlated with post mortem data and changes in neutrophil and platelet behaviour previously established during shock.

Table 1

Organ	Mean albumin leak index + SD ($x10^{-2} \times min^{-1}$) (n=6)			
	Controls		*E.coli* shock	
Lungs	-0.27±0.10	(-0.56±0.45)	0.56±0.89	(0.99±0.39)
Liver	-0.09±1.98	(0.46±0.42)	1.76±0.39	(2.30±0.60)
Abdomen	0.34±0.17	(0.42±0.38)	3.17±0.80	(3.70±0.23)

Values in parentheses obtained from analyses over 4 hours only

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AUTOMATIC CONTROL OF THE DEPTH OF ANESTHESIA DURING TRAUMATIC SHOCK EXPERIMENTS. Z. Khakpour, G. Schlag and J. Davies*. Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria; * Roodeplaat Research Lab., Pretoria, South Africa

Introduction: The effect of anesthetics on hemodynamic and/or the autonomous nervous system depends on the type and depth of anesthesia. Based on an already established device, we have attempted to develop a more advanced automatic controlling system to (a) standardise the conditions of anesthesia, (b) minimise further operator interaction and control the continuous infusion rate of pentobarbital sodium by EEG-adjusted parameters.

Methods: Following initial administration of anesthesia by pentobarbital and as soon as baselines for hemodynamic parameters (e.g. cardiac output 120 - 150 ml/kg BW) have been reached, the servo-mechanism system is started, requiring no further operator interaction. The EEG signal is digitalised at a sampling rate of 1,000 S/s for about 2 seconds at intervals of one minute. All DC-offsets are removed and a Fast Fourier analysis is performed by using a window function (Hanning). This is followed by calculation of the power spectrum. The first moment of the power spectrum in the range of 2 to 16 Hz is subject to PI closed-loop control as an actual value. As an actuator, we use an infusion pump (B. Braun) controlled via a RS-232 interface. This control loop may only be employed as soon as a "learn" phase has been concluded. The control is automatically switched off beyond a pre-established expiratory CO_2 threshold level. The system is manufactured as a "stand alone" device.

Conclusion: With this servo-controlled mechanism of anesthesia the mean supply of pentobarbital sodium was minimised to 1.5 - 2.0 mg/kg BW.

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PULMONARY DAMAGE AFTER RECURRENT ADMINISTRATION OF ENDOTOXIN OR ENDOTOXIN AND ZYMOSAN ACTIVATED PLASMA - A SHEEP MODEL. H.-C. Pape, A. Dwenger#, G. Regel, D. Remmers, G. Schweitzer#, and J.A. Sturm (Spon: H.J. Oestern).
Departments of Trauma Surgery and Clinical Biochemistry#, Medical School Hannover, F.R.G.

Pulmonary failure is one feature of the posttraumatic multiple organ failure (MOF), supposedly caused by hyperactivated granulocytes (PMNL) and alveolar macrophages (AMϕ). Merino sheep (25-30 kg) were given bolus injections of E.coli endotoxin (ET; 1 µg/kg b.w.; group 1; n≤20) and E.coli endotoxin + zymosan activated plasma (0.75 µg/kg b.w. + 20 ml ZAP; group 2; n≤7) every 12 hours for 5 days. Blood and bronchoalveolar lavage fluid (BAL) were obtained before the first (day 1), fifth (day 3) and nineth (day 5) endotoxin injection. Systemic O2-partial pressure (paO2 as % of day 1), difference of pulmonary artery pressure before and after ET (ΔPAP; mm Hg), epithelial lining fluid (ELF)/plasma ratio of albumin (R), blood-PMNL and BAL-AMϕ chemiluminescence (CL; as % of day 1 value), and the fraction of BAL-PMNL (%) were determined. * p<0.05, Student's nonpaired t-test.

		R		CL-PMNL(%)	CL-AMϕ(%)	%BAL-PMNL		ΔPAP		paO2(%)	
		day1	day5	day1/ 3/ 5	day1/ 3/ 5	day1	day5	day1	day5	day1	day5
group 1	$\bar{x}$	0.05	0.08	100/150/75	100/209/123	2.0	19.6	27	5	100	106
	SEM	0.01	0.01	7/ 22/13	40/ 23/ 29	0.6	6.2	1.4	1.7	3	4
group 2	$\bar{x}$	0.08	0.29*	100/145/79	100/ /393	2.0	5.6*	16*	5.5	100	95*
	SEM	0.06	0.07	17/ 21/16	42/ / 21	1.3	1.4	2.6	2.3	2	3

In conclusion, despite increased hemodynamic reaction (ΔPAP) after ET in group 1, more pulmonary damage (R, paO2 of day 5) was seen in group 2 receiving additional ZAP, but less ET. While the CL responses of blood-PMNL did not differ for both groups, the AMϕ-CL response was higher for group 2 indicating that additional ZAP triggers lung damage processes presumably by an altered AMϕ activity. BAL-PMNL invasion seemed to be a result but not the cause of permeability increase.

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INDUCTION OF MULTIPLE ORGAN FAILURE (MOF) IN A STANDARDIZED SHEEP MODEL G.Regel[1], A.Dwenger[2], H.C.Pape[1], G.Schweitzer[2], D.Remmers[1], J.A.Sturm[1] (Spon: H. Redl) Depts. of Trauma Surgery[1] and Clinical Biochemistry[2], Medizinische Hochschule Hannover,FRG

MOF is nowadays accepted as the most frequent complication following multiple trauma. In order to study related pathomechanisms, our aim was to find a standardized experimental model that would accurately imitate clinical MOF.

The 1st model (GrI) represents the combined insult, applying both endotoxin (ET) and Zymosan activated plasma (ZAP) (0.75µg/kg BW + 20ml ZAP, n=7). The 2nd form of injury (GrII) is a combination of initial hemorrhagic shock (2h mean art. pressure ≤50mmHg) and in addition the same insult as in GrI. The experiment was performed for 5 days, measuring representative organ function parameters (listed for the 1st, 5th and 10th day).(*p≤0,05 non paired Student t-Test)

		Lung (albumin)			Heart	Liver	Kidney	BAL
		R(ELF/plasma)	PAP $\bar{x}$(mmHg)	paO_2 %	CI(L/min.·m²)	SDH(U/l)	Crea(mg/dl)	% PMNL
GrI	1	0.08	14.3	100.0	4.1	8.5	0.8	2.0
	5	0.29	16.0	95.3	5.2	6.2	0.7	5.6
	10	0.09	16.1	104.2	6.7	4.1	0.7	7.4
GrII	1	0.08	15.1	100.0	4.1	5.4	0.9	0.8
	5	0.48	15.8	96.3	4.2*	10.2	0.8	33.0*
	10	0.58	19.1*	84.9*	4.6*	166.2	1.2	12.1

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Specific signs of single organ failure were demonstrated in Gr.I. Here however the changes were transient and an adaption to the induced toxins (ET/ZAP) was seen. In Gr.II, the combination of initial hemorrhagic shock and bolus injection of ET and ZAP over 5 days lead to permanent changes of organ function. From these results we feel that the model used in Gr.II is most appropriate to imitate the clinical course of MOF and should be applied for further investigations.

LUNG INJURY AFTER PSEUDOMONAS INFUSION: DIRECT AND INDIRECT HEMATOGENOUS BACTERIAL EFFECTS. 179

HM Loick, MD, DJ Dehring, MD, LD Traber, RN, R Tokyay, MD, DL Traber, PhD, U TX MED BR, SHRINERS BURNS INST, GALVESTON, TX, USA. (funded by SBI 15872 & HL 36286) Sepsis-induced pulmonary injury may be caused by both hematogenous mediators or direct bacterial toxicity. Relative roles were determined by infusing live *Ps. aeruginosa (Ps.)* in the left pulmonary artery and comparing the changes between contralateral lungs in awake sheep. The left pulmonary artery was chronically cannulated with a 22 G catheter and also surrounded with an ultrasonic flow probe.. *Ps.* (4 - 29 x 10^8) were infused into the left pulmonary artery over 15 min (n=5, *Ps.*GR) or saline was infused (n=5, Sham). The lungs were harvested 24 hours after *Ps.* infusion for wet/dry lung weight ratio and tissue conjugated dienes (CD) determination. **RESULTS:** *Ps.* in arterial blood were negligible during *Ps.* infusion and not detectable after 15 min. *Ps.* dramatically increased vascular resistance in the left lung (LL), with an increase in right lung (RL) delayed to 1 hr. Neutrophils decreased from 5335±916 to 1284±450 cells/µl during the first hour. CD were significantly and paradoxically higher in RL (2.8±0.5) compared to LL (1.2±0.2) of the *Ps.*GR but not compared to Sham RL (2.0±0.1). The LL wet/dry ratio of *Ps.*GR (5.3±0.2) was not different to its RL (4.8±0.2), but higher compared to Sham LL (4.3±0.2). The RL wet/dry ratio in *Ps.*GR was not significantly increased compared to the RL of the Sham animals.

PULMONARY VASCULAR RESISTANCE INDEX AFTER *Ps* INFUSION (dynes sec cm^{-5}/m^2)

Time (hour)	baseline	0.25	0.5	1	2	8	24
Left Lung (x 10^3)	0.98±0.12	8.96±3.1*†	9.17±2.98*†	5.45±1.17*†	2.60±0.90	1.29±0.30	1.14±0.14
Right Lung (x 10^3)	0.28±0.05	0.26±0.04	0.31±0.04	0.58±0.11*	0.37±0.05	0.32±0.04	0.25±0.03

Data are mean±SE, *p<0.05 vs baseline (Dunnett's test), †p<0.05 between left and right lung (t-test)
CONCLUSION: The delayed increase in RL pulmonary resistance is probably due to release of mediators from the *Ps.* infused left lung, rather than direct bacterial effect, since only minimal *Ps.* were detected systemically. Edema was present only in LL and probably related to an direct bacterial effect. The mediators, which caused the different CD levels between the bacteria infused and contralateral lung, are unknown.

HEMOGLOBIN AS A MODULATOR OF LYMPHATIC PUMPING FOLLOWING TISSUE INJURY AND SHOCK. 180

M.G. Johnston, R. Elias, G. Wandolo, J. Eisenhoffer
Trauma Program, Sunnybrook Health Sciences Centre, Toronto, Ontario Canada M4N 3M5

Cell-free hemoglobin appears in sheep lymph following tissue injury or after the systemic administration of endotoxin. We previously demonstrated in vitro that purified hemoglobin inhibits lymphatic pumping by depressing the sensitivity of the vessel to changes in transmural pressure. The purpose of this study was to test the effects of hemoglobin in vivo. We utilized a sheep model that allows the quantitation of pumping activity without the complication of variable lymph inputs. A segment of the mesenteric lymphatic vessel was isolated from the lymph input by placing a catheter in the direction of flow close to the point where it emerges from the terminal mesenteric node and inserting a second catheter downstream from the node (10 to 15 cm) against the direction of flow. All of the tributary vessels were tied off and a fluid reservoir containing Krebs was the only input to the lumen of the duct. The blood and nerve supplies to the vessel were left intact. All experiments were performed with conscious animals. A transmural pressure was applied to the vessels to stimulate pumping. The addition of autologous oxyhemoglobin into the lumen of the vessel ($5X10^{-5}$M) resulted in complete inhibition of pumping activity. With the lymphatic ducts isolated from lymph input and the hemoglobin administered into the systemic circulation (final plasma concentration $5X10^{-6}$M), pumping was not inhibited (the absence of hemoglobin in the lumen was confirmed spectrophotometrically). Therefore, it appeared that hemoglobin suppressed pumping only when present in the vessel lumen suggesting that the lymphatic endothelium regulated this response. These results suggest that hemoglobin may be an important modulator of lymphatic pumping following vascular injury or sepsis and also point to a dynamic role for the lymphatic endothelial cell in regulating lymphatic contractile activity.

OLEIC ACID CAUSES A DOSE-DEPENDENT PERMEABILITY CHANGE IN SHEEP. 181

S. Bergdahl*, A. Larsson*, L. Smith*, B.Risberg.
Dept surgery, Östra sjukhuset, Univ. Göteborg, Sweden.

We have previously demonstrated that oleic acid increased pulmonary microvascular permeability as measured by reduction in the osmotic reflection coefficient. The aim of the present study was to evaluate any relation between the dose of oleic acid given and the microvascular lesion as measured by extravasation of radiolabelled transferrin. Sheep, anesthetized and mechanically ventilated, were given different doses of oleic acid (0,005-0,05 mg/kg BW)i.v. Two groups of control sheep were used (with and without pulmonary artery catheter). All experimental animals had arterial and venous lines and a pulmonary artery catheter. Erytrocytes were labelled with Tc-99m and transferrin with In-113m. Leakage was calculated as microvascular to interstitial macromolecular flux (coefficient of transfer, "alfa") according to Gorin et al (1), and as transferrin leake index

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(TLI) according to the simplified model of Dauber (2). Lung capillary preassure (Pc) was measured according to Holloway et al (3). The animals were monitored for 4-6 hours.

Results:		alfa$_4$	TLI$_4$	n
oleic acid	0,05 mf/kg BW	(10^{-4} min^{-1})	(10^{-4} min^{-1})	
		63 ± 17	226 ± 85	9
" "	0,02	32 ± 6	165 ± 25	8
" "	0,005	17	110	1
control + PA catheter		7,7 ± 0,7	61 ± 10	3
Control		1,2 ± 0,05	9 ± 5	4

Pc was unchanged in all groups during the experiment. Oleic acid enhanced transcapillary solute flux dose dependently at normal capillary pressure.

Ref: 1. Gorin et al. J. Appl. Physiol. 45:225 1978 2. Dauber et al. J. Appl. Physiol. 59:564,1985.
3. Holloway et al: J. Appl. Physiol. 54:846, 1983

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A MODEL OF RECURRENT ENDOTOXEMIA: HEMODYNAMIC, BIOCHEMICAL, MORPHOLOGICAL, AND COMPUTER-TOMOGRAPHY RESULTS. **B. Klosterhalfen, K. Hörstmann-Jungemann, Ch. Müller-Leisse, CJ. Kirkpatrick. Inst. of Pathology and Radiology, The Technical University of Aachen, 5100 Aachen, West-Germany**

Endotoxemia was induced in domestic pigs by intravenous application of an E. coli endotoxin (LPS; WO111:B4). The experiment was continued for a maximum of 18h. Endotoxin-infusions were administered at the time points 0h, 5h, and 10h in a dose of 0.5µg/kg over a period of 30 min. Hemodynamic data were obtained every 15 min over the whole experimental duration. Within the first 60 minutes of each LPS application TxB_2, 6-keto-$PGF_{1\alpha}$, TNF-α, LTC4, - D4, -E4, and IL-6 plasma levels were measured at intervals of 15 min, followed by intervals of 60 min. CTs of the lungs were prepared at various time points during the experiments. After 18h the animals were killed by hyperkalemic cardiac arrest, the lungs were immediately removed and prepared for routine light and transmission electron microscopy.

Hemodynamic variables after the first LPS infusion were characterized by increased PAP, decreased SAP and and a hypodynamic cardiac output. TxB_2 plasma levels during this period peaked first, followed by TNF-α, the leukotrienes, 6-keto-$PGF_{1\alpha}$, and IL-6. Additionally, this phase of recurrent endotoxemia presented high TxB_2/6-keto-$PGF_{1\alpha}$ ratios which could be a specific explanation for the registered hemodynamic changes. The hemodynamic response to the second and third LPS applications were more moderate. No characteristic chronological order of the mediator plasma peaks and striking, high 6-keto-$PGF_{1\alpha}$/TxB_2 ratios could now be detected. CT and morphological findings were similar to the alterations found in early human ARDS, such as severe interstitial edema, broadened alveolar septa and endothelial cell damage.

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SITE OF BACTERIAL INFUSION ALTERS MORTALITY AND PATHOPHYSIOLOGIC RESPONSE TO E.COLI SEPSIS IN IMMATURE SWINE. P.A. Lee, F. K. Straughn, R. W. Pryor and J.R. Matson. Humana Hospital-Medical City Dallas, Dallas, TX 75230.

The aim of these studies was to determine if site of bacterial infusion (e.g., peripheral vs. central vein) alters morbidity and mortality in an immature swine model of E. coli-induced sepsis. 12 anesthetized pigs (4-6 weeks old) were surgically prepared with femoral and jugular venous catheters, a femoral arterial catheter and a saphenous vein catheter. All animals received a two hour infusion of live E. coli (1 x 10^9 CFU/kg) via either the jugular (Group 1, n=6) or the saphenous (Group 2, n=6) vein. Animals were monitored for 10 hours and observed until death. In both groups of animals, E. coli infusion produced sustained increases in heart rate,respiratory rate and mean pulmonary arterial pressure and the characteristic leukopenia and thrombocytopenia seen in adult animal models of E. coli sepsis. Group 1 and Group 2 animals also had sustained hypoglycemia following bacterial infusion. The striking differences between the two groups were:(1) severe lactic acidemia in Group 2 animals (7.8 ± 0.7 mEq/L) vs. Group 1 (3.3 ± 0.6 mEq/L), (2) all Group 1 animals survived the 7 day observation period while 5 of the Group 2 animals died within 13 hours of the bacterial infusion and 1 survived 7 days, and (3) Group 2 animals had more severe and widespread organ damage at necropsy than Group 1 animals. The only lesions found in Group 1 animals were marked pulmonary edema, hemorrhage and atelectasis. Group 2 animals had similar pulmonary lesions in addition to moderate hemorrhage and necrosis of the kidneys and adrenal glands, splenic hemorrhage and severe intestinal damage with hemorrhage and numerous intussusceptions. Differences in mortality and histopathology between animals receiving central vs peripheral infusions in this model may be due to longer systemic residence time of bacterial in the latter allowing for greater diffuse damage to occur. First pass pulmonary clearance of bacteria may be more important in the former; moderating the systemic impact of this bacterial challenge.

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SYSTEMIC AND INTESTINAL OXYGEN TRANSPORT PARAMETERS FOLLOWING ACUTE NECROTIZING PANREATITS IN SWINE. C.D. Schwarz, W. Hasibeder, M. Haisjackl, H. Sparr, G. Klima, M. Herold, N. Salak, M. Seyr, B. Abendstein.
Univ. Innsbruck, Departments of Surgery, Anesthesiology, Internal Medicine and Histology, A-6020 Innsbruck.

The effects of an acute necrotizing pancreatitis (P) on systemic (VO_2) and intestinal oxygen transport parameters (DO_2, VO_2, mucosa pO_2 and pH) were investigated in 5 pigs (P) as compared with 4 control animals (C). Systemic DO_2 was kept constant in both groups by infusion of cristalloids at a rate of 70 ml/kg BW*hour. The animals were anesthetized (sufentanyl, midazolam), paralyzed and ventilated with oxygen in air. A segment of the jejunum of 200 to 300 cm in length was isolated and autoperfused via the femoral artery, flow was measured by an electromagnetic flow probe. The pO_2 of the exposed mucosa was continuously recorded using a multiwire Clark-type electrode. Mucosa pH was determined by intraluminal pCO_2 tonometers. Pancreatitis was induced by injection of taurocholic acid into the main pancreatic duct. **Results:** Severe necrotizing pancreatic lesions were found at autopsy in all animals of the P-group. Systemic and intestinal oxygen transport parameters remained stable throughout the 4 hours observation period and there were no significant differences of these variables between both groups. Baseline intestinal oxygen transport parameters were: Blood flow 48.5±12.8 (C) vs. 59.9 ±15.8 (P) ml/100g*min; DO_2 5.2±1.6 (C) vs. 6±1.4 (P) ml/100g*min; VO_2 1.3±0.5 (C) vs. 1.7±0.2 (P) ml/100g*min; Mucosa pH 7.2±0.06 (C) vs. 7.21±0.04 (P); Mucosa pO_2 34.5±12.3 (C) vs. 36.2±9.7 (P) mmHg. **Conclusions:** *Continuous and aggressive fluid loading prevents* alterations in systemic and splanchnic oxygen transport parameters in the early phase of an acute necrotizing pancreatitis.

This study was supported by Laevosan Inc. Austria

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CHEMILUMINESCENCE RESPONSE OF WHOLE BLOOD, POLYMORPHONUCLEAR LEUCOCYTES AND MONOCYTES FOLLOWING EXPERIMENTALLY INDUCED HAEMORRHAGIC NECROTISING AND EDEMATOUS PANCREATITIS. S. Albrecht, T. Zimmermann, R. Schuster, T. Freidt, H. Hilbrich
Medical Academy Dresden, Dresden, O-8019, Germany

The formation of reactive O_2-species in the pancreas of pigs after induction of necrotising or edematous pancreatitis was studied by means of sensitised chemiluminescence (Luminol, Lucigenin), cytoflowmetry and immunofluorescence technique. The chemiluminescence analyses of whole blood show a rather variable course. The respiratory burst of the granulocytes develops with varying intensity reaching its maximum 1.5-2 hs. after the induction of pancreatitis, whereas the CL-response of stimulated monocytes (within an lymphocyte/monocyte pool) increases at first very slowly, then shows a sharp increase after 2 hs. and reaches its maximum within 4 hs. after induction of pancreatitis. The results obtained are discussed in the light of cytoflowmetric results and with respect to a possible specification of quite definite O_2-radicals.

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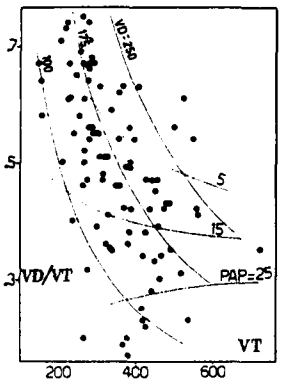
THE NATURE OF EARLY RESPIRATORY CHANGES IN ENDOTOXIC SHOCK. I.Giovannini, T.Emanuele*, C.Chiarla*, M.Castagneto*, M.Cagossi*, G.Nanni*, A.Borzone*, D.Gui*, L.Tazza*, G. Boldrini*, Shock Center CNR, Clin.Chirurg.Catholic Univ.(Prof.F.Crucitti) 00168 Rome Italy

Early respiratory changes were examined in 103 measurements in 13 dogs with endotoxic shock. Total and alveolar ventilation (VE, VA, L/min/m^2), and pulmonary shunt (%) were determined with tidal volume and dead space (VT, VD, ml/m^2), wasted ventilation (VD/VT), ventilation/perfusion ratio (V/Q), cardiac index (CI, L/min/m^2), pulmonary artery pressure (PAP, mmHg), base excess (BE, mEq/L) and other variables. After endotoxin, VE, VA, VD/VT and V/Q increased, shunt decreased ($p<0.01$ for all). In spite of slightly declining VCO_2, VE rose as a function of increases in VA and VD, which were in turn mediated by the effect of decreasing BE, and by the interaction between VT and decreasing PAP:
$VA=-.0013(BE)(VCO_2)+.0142(VCO_2)-.1352$; ($r^2=.7$, $p<.001$)
$VD=.33(VT)-5.14(PAP)+103.7$; ($r^2=.5$, $p<.001$) (figure).
Shunt decreased as a function of increasing V/Q, which resulted from the combination of falling CI with BE-mediated hyperventilation: $V/Q=3.62/CI-.22(BE)-1.64$; ($r^2=.7$, $p<.001$). Early respiratory changes in endotoxic shock are in part an indirect consequence of hemodynamic and metabolic interactions. This must be accounted for when assessing the responsibility of direct toxic effects of endotoxin on the lungs.



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EFFECTS OF INCREASED THORACIC DUCT LYMPH FLOW RATE ON THE EXTRAVASCULAR LUNG WATER

Takeshi Iwata, Ill Sung Kim, Hiroshi Noguchi and Yoshiaki Takumi. Department of Anesthesiology and Acute Medicine, Aichi Medical University, Nagakute-cho, Aichi, 480-11, Japan

Impairment of lymphatic drainage from the lung has been considered as one of the causes of the pulmonary edema. The purpose of this study is to confirm if an increase in lymphatic flow rate in the left lymphatic duct can cause a development of pulmonary edema. The development of pulmonary edema was assessed as an increase of the extravascular lung water (EVLW) determined with the gravimetric method after Pearce et al. The study was performed with the anesthetized mongrel dogs. They were thoracotomized in the 6th intercostal space and the left lymphatic duct was laid open at the height of 9~10th thoracic vertebrae. The lymphatic duct was ligated and cannulated to the cranial direction with a polyethylene catheter. The dogs were divided into 5 groups. The first group is the Control and received the sham operation and lactated Ringer's solution (LR) intravenously with a rate of 10 ml/kg/hr. The second group received LR infusion of 25 ml/kg/hr i.v.. In the third group, the left atrial pressure was elevated to 20 mmHg by inflating the balloon in the left atrium and LR infusion of 10 ml/kg/hr i.v.. The fourth group received LR infusion of 10 ml/kg/hr i.v. and that of 15 ml/kg/hr into the left lymphatic duct. The fifth group was the same as the fourth group except left atrial pressure elevation up to 20 mmHg. All groups were studied for 5 hours and at the end, EVLW was determined. PaO_2 decreased with time in all groups and statistically significant falls were observed only in the 4th and 5th groups. In EVLW, no significant difference was found in 1~4 groups. The EVLW in the 5th group was increased siginificantly. We conclude, an increase in left lymphatic flow rate facilitates a formation of lung edema.

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DOSE AND ENTRY PORT RELATED EFFECTS OF ENDOTOXIN ON KIDNEYS

K.Kürten,Ferdinand-Sauerbruch-Hospital,Wuppertal,Dep.of Surg.,FRG;

In spite of intensive investigations systematic and reproductive data on the effects of various endotoxin concentrations and its diverse ports of entry to organism are extensively missing. Since kidney is very sensitive to endotoxicosis,in serial investigations changes in function and metabolism of the dog kidney were investigated after intraperitoneal(i.p.),intravenous(i.v.)and intermittend intramuscular(i.m.)injection of E.coli endotoxin(o.2-2mg/kg).Method:Narcotized mongrel dogs got i.p.,i.v.or i.m.injections of several doses of endotoxin.Afterwards kidney function was followed for 4 hrs.to 1o days.Then the kidneys were removed and cortex of ATP, ADP,AMP(TAN),lactate,pyruvate and glucose was measured.Resultes:After i.p. endotoxin application kidney function was significantly reduced at doses at least of o.75mg/kg.TAN-content of kidney cortex could be closely correlated to the amount of endotoxin injected.Minor doses did not show an effect neither on function nor on metabolism.The i.v.injection of 2mg/kg endotoxin leads to an extensive reduction of the initial kidney function. About 5o% of the kidneys ceased function within the first 4o minutes. During this time a progredient loss of TAN and marked changes of its components could be observed.After i.m.apply of daily o.2mg/kg endotoxin during 1o days(2.omg/kg in all)the reduction of kidney function is also significantly diminished in comparison to the control group.Indeed metabolic data show no significant differences in total TAN,but a high conversion rate of the more energetic ATP to the less energetic AMP could be seen.This means, that the organism is not getting used to a fragmentary application of small doses of endotoxin like otherwise reported.

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A CLINICALLY RELEVANT MODEL OF MULTIPLE ORGAN FAILURE IN THE RABBIT.

Sen Hu*, Zhi-Yong Sheng*, Mui-Min Tian* (Spon: Zheng-Yao Luo). 304th Hospital, Beijing 100037, China.

We used clinical markers of MOF in our rabbit model: 1) more than two organ function failures 2) characteristic metabolism, 3) failure of gut mucosal barrier to bacteria, and 4) histopathologic alterations in multiple organs. Animal in test group (T) were fed live E coli (2x10E11/kg), followed by hemorrhagic shock (40 mmHg BP for an hour) plus rapid resuscitation with shed blood and Ringer'lactate. Five control groups were used: sham operation (C1), fed live E coli only (C2), shock with resuscitation (C3), shock without resuscitation (C4) and to investigate the effect of endotoxin (C5) fed with dead E coli. After resuscitated from shock the animals in T continued to developed gut failure (bacteremia and endotoxemia), hyperdynamic and metabolic responses on first three days, and progressively followed by MOF and death. Mortality rate: T (85.0%)>C4 (66.7) >C5 (33.3%) >C3 (13.3%). Animals with MOF: T (71.0%) >C5 (16.7%)>C4 (13.3%) >C3 (6.7%). Results for animals with MOF in T are tabulated below:

	Day	B	1	3	5	7
SGPT (ALT)	(IU/L)	38±5	187±24*	563±200*	352±70*	89±13*
PaO2	(mmHg)	96±5	80± 7*	60± 10*		97± 8*
Platelets	(10 /l)	170±17	120±20*	50±14*		146±21
Creatinine	(mg %)	1.4±0.1	2.0±0.3*	3.7±0.4*	2.2±0.5	
Phenylalanine	(umol/l)	80±15		121±24*		
Positive Blood Culture (CFU)	(%)	5.5	82.5*	62.5*		12.5

Mean ± SD, B = Baseline, * P < 0.01 Compared to baseline (t test).

Histopathology showed lung edema, hemorrhage, and inflammatory cell infiltrate, the kidney exhibited tubular injury, and the liver showed parenchymatous degeneration and necrosis. We conclude that this stable model reproduces multiple clinical characteristics of MOF and offers opportunity for the study of mechanisms and therapy.

RELATIONSHIP BETWEEN LIPID PEROXIDATION AND PROGRESSIVE LUNG AND LIVER DYSFUNCTION CAUSED BY BACTERIAL INDEPENDENT PERITONITIS. **190**

C. LaLonde, J. Knox, Y.K. Youn, R. Demling. Harvard Medical School, Boston, MA, 02115.

Our purpose was to determine the relationship between acute peritonitis, oxidant induced lipid peroxidation (LP), and multiple (lung, liver) organ dysfunction. 12 male Wistar rats were given IP Zymosan A (.75g/g) in mineral oil and fluid resuscitated. Half were given cefoxitin (C). Animals were killed at 24 hrs (6) and 9 days (6) and compared to controls (6). Lung and liver LP was measured as malondialdehyde (MDA).

	HCT %	TOTAL PROTEIN g/dl	PaO_2 (torr)	LUNG MDA nMol/g	LIVER LDH u/L	LIVER CATALASE k	LIVER MDA nMol/g	LIVER SGOT u/L	WT LOSS g
Control	39±2	6.8±3	88±8	67±5	252±43	101±28	44±12	87±21	0
24 Hrs	46±2*	5.7±.3*	91±22	90±20	227±54	49±5*	51±14	84±10	1±6
9 Days	35±5	6.7±.5	62±20*	187±52*	621±278*	31±5*	56±7	174±82	47±14*

*=significantly different from controls p<.05

Severe sepsis was evident at 24 hrs in (C) and non (C) animals, the latter growing gr-rods, not seen with (C). Animals recovered but remained chronically ill. IP cultures at 9 days were all negative. Lung and liver dysfunction was absent at 24 hrs, but histology demonstrated neutrophil sequestration. No oxidant changes were noted. At 9 days, severe lung dysfunction was noted along with a 250% increase in MDA. Lung infiltration with mononuclear cells and fibrosis was noted. Also, hepatomegaly and vacuolization of parenchyma as well as an increase in liver enzymes, but no increase in MDA was noted, despite a decrease in liver catalase. We conclude that: (1) organ failure progresses even after the septic episode resolves, and (2) lipid peroxidation is evident in the lung, but not the liver injury.

EFFECT OF ENDOTOXOCOSIS ON TISSUE AND PLASMA LEVELS OF CALCITONIN GENE-RELATED PEPTIDE LEVELS. **191**

N. Aiyar, E. Griffin, E.F. Smith III. SmithKline Beecham Pharmaceuticals, plc, Dept. of Pharmacology, King of Prussia, PA.

Calcitonin gene-related peptide (CGRP), a 37-amino acid peptide, has been identified in the central and peripheral nervous system, particularly in nerves supplying blood vessels. Previous studies have reported that endotoxemia was associated with increased plasma CGRP concentrations in a dose and time dependent manner (Wang et al., Circ. Shock 31:49, 1990). The purpose of this study was to investigate the changes in tissue content and plasma concentrations in male Sprague-Dawley rats injected with a non-lethal dose of 3 mg/kg (i.v.) E. coli endotoxin (LPS). Plasma CGRP concentrations, measured by RIA, were initially 81.9±22.7 pg/ml, and were increased to 273±38.4 pg/ml 2 hr after induction of endotoxemia (n=8;p<0.001). CGRP levels in aorta, vena cava and mesenteric artery were initially 6.2±2.4 pmol/g, 6.4±1.3 pmol/g and 8.6±1.7 pmol/g, respectively, and 2.8±0.9 pmol/g, 11.4±2.8 pmol/g, and 11.8±2.8 pmol/g 2 hr after LPS. In left ventricle, lung and kidney, CGRP content increased from 0.4±0.1 pmol/g, 1.5±0.3 pmol/g, and 0.3±0.1 pmol/g to 0.7±0.2 pmol/g, 1.9±0.3 pmol/g, and 0.9±0.4 pmol/g, respectively. CGRP content of stomach (7.3±1.9 pmol/g initially vs. 6.2±1.6 pmol/g at 2 hr) and duodenum (9.1±1.6 pmol/g initially vs. 7.4±1.2 pmol/g at 2 hr) were unchanged following endotoxemia. These results indicate that there are considerable regional differences in tissue CGRP content in the rat. Moreover, endotoxemia produced significant increases in plasma CGRP concentrations, and elevations in left ventricular, lung, and kidney tissue content. However, there were no changes in CGRP content of vena cava, mesenteric artery, stomach or duodenum tissue content, although a decreased CGRP content in aortic tissue was observed. The changes in plasma concentrations and tissue content of CGRP, and the contribution to homestatic or pathophysiologic responses of endotoxemia, remains to be elucidated.

ESTABLISHMENT OF BLOOD CHEMISTRY AND ENZYME CONCENTRATIONS IN THE CONSCIOUS RAT AND THEIR CHANGES DURING DOCUMENTED ENDOTOXIC SHOCK **192**

D. J. Brackett, R. D. Passey*, M. R. Lerner*, T. J. Lander* and M. F. Wilson, VAMC and Depts. of Surgery, Pathology, Anesthesiology, and Medicine, University of Oklahoma HSC.

Blood chemistry and enzyme concentrations are used clinically as diagnostic tools to guide decisions determining the therapeutic regimen for multiple disease processes. However, not even baseline values of these clinically routine blood measurements have been established in conscious animal models. In this study blood samples (1 ml) were taken to determine basal values in 28 **conscious**, instrumented, freely moving rats and again 4 hrs after 20 mg/kg endotoxin (n=14) or saline (n=14). Documentation of endotoxic shock included a transient 30 min hypotensive episode, decreased cardiac output, and increased heart rate and systemic vascular resistance. Hemorrage of the small intestine was found in all endotoxic animals. Non-fasting basal **blood chemistry** values were sodium = 141 ± .4, potassium = 3.8 ± .1, chloride = 105 ± 0.4, and CO_2 = 25 ± .5 **mEq/L**; total protein = 5 ± .06, albumin = 2.8 ± .03, and uric acid = .8 ± .06 **gm/dl**; and phosphate = 7 ± .1, glucose = 136 ± 4, total bilirubin = .15 ± .02, cholesterol = 62 ± 1, triglycerides = 32 ± 4, calcium = 10 ± .05, blood urea nitrogen = 17 ± .7, and creatinine = .2 ± .01 **mg/dl**. Basal **blood enzymes** were alkaline phosphatase = 280 ± 8, SGOT = 120 ± 4, SGPT = 59 ± 2, gamma-GT = 9 ± .1, total CK = 327 ± 32, CK1 = 146 ± 17, CK2 =

Notes

108 ± 20, CK3 = 69 ± 9, LDH = 398 ± 58, LDH1 = 15 ± 2, LDH2 = 21 ± 3, LDH3 = 27 ± 5, LDH4 = 38 ± 7, and LDH5 = 340 ± 64 **U/L**. Four hours after endotoxin (ETX) and saline there were significant differences between groups for all blood chemistries except total bilirubin and uric acid. Every enzyme except alkaline phosphatase was several fold higher after ETX. This data establishes: **1)** basal chemistry and enzyme levels for the conscious rat, **2)** that ETX induces dramatic changes in these levels indicating significant multiple organ dysfunction and cellular damage, and **3)** a data base to determine the efficacy of therapeutic interventions during ETX shock.

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HISTOPATHOLOGICAL FINDINGS OF ORGANS IN MICE AFFECTED BY ENDOTOXIN, PAF OR TNF. H. Ogata, T. Takiguchi, X. Luo and Z. Chi, F. Ishitobi*, K. Iidaka*.
Dokkyo Univ., Dept. of Anesthesia, Dept. of Pathology*, Mibu, Tochigi, Japan, 321-02

(Acute experiment) Histopathological findings of lung, heart, liver, spleen, kidney, small intestine stained with hematoxylin-eosin were investigated in mice (C3H/HeN) injected intravenously with endotoxin (1 mg/kg), PAF (2.5 ug/kg) or TNF (1 mg/kg), respectively. Five mice were used for each drug in acute and chronic experiment. Endotoxin: Lung (intraalveolar hemorrhages), heart (waxy necrosis), liver (congestions, fatty degeneration), spleen (congestions), kidney (congestions). PAF: Lung (intraaveolar hemorrhages), liver (fatty degenerations, congestions, cellular degeneration), kidney (glomerular capillary dilation, congestion), spleen (congestion, polymacrophages). TNF: Heart (waxy degenerations), liver (fatty degenerations), kidney (congestion). (Chronic experiment) Each mice was injected i.p. with endotoxin (0.75 mg/kg), PAF (7.5 ug/kg), TNF(0.5 mg/kg), respectively for 7 days. (Results) Endotoxin: Lung (hemorrhages, congestion), liver (neutrophiles, lymphocytes invasion, necrosis of liver cells, dropped fatty metamorphosis, centrolobular congestion), spleen (macrophages & neutrophiles invasion, enlargement of spleen), kidney (congestion. Spleen/body weight co-efficient was 0.01, although normal spleen/body weight was 0.003. PAF: Lung (intraalveolar hemorrhages, hyalinosis, congestions), liver (congestions), spleen (congestions) TNF: Lung (intraalveolar hemorrhages), liver (fatty degenerations), heart (waxy necrosis), spleen (congestions). (Conclusion) Endotoxin, PAF and TNF revealed similar pathological findings in each organ in acute experiment. The characteristic findings were hyalin degeneration of the lung in PAF and waxy necrosis in TNF although there were few findings in kidney and small intestine.

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A ROLE OF NEUTROPHIL ELASTASE-ALPHA 1 ANTI-TRYPSIN COMPLEX IN STORED BLOOD IN PULMONARY INVOLVEMENTS AFTER MASSIVE TRANSFUSION O. Umegaki, M. Aibiki, K. Honda, S. Ogura, Y. Shirakawa and K. Ogli. Kagawa Med. Sch., Dept. of Anesthesiology & Emergency Med. Kagawa, 761-07, Japan.

We studied changes in neutrophil elastase (NE)-alpha 1 anti-trypsin complex (EAC), using an enzyme-linked immunosorbent assay, in stored blood donated by 7 volunteers. The withdrawn blood was stored in bags with citrate-phosphorus-dextorose solution and preserved in a refrigerator at 4 C. EAC in the stored blood remained at normal levels on the 3rd day of storage but increased significantly by the 14th day, and had further increased at the 21st day of storage. These results indicate that EAC in stored blood may elevate in parallel with the preservation period. Generally, EAC is thought to be inactive in vivo. However, it has recently been suggested that EAC may activate macrophages, resulting in tissue damage. We thus designed the following experiment to define the role of the increased EAC in stored blood in pulmonary involvements after massive transfusion. Japanese white rabbits were divided into EAC- and saline-injected group. EAC was produced from 5 ug of NE and 50 ug of alpha 1-antitrypsin in saline. Following injection, interstitial lung derangements were revealed by microscopic examination in the EAC group, whereas no pathological changes were found in the saline injected group. These results indicate that the increased EAC in stored blood may have a role in pulmonary involements after massive transfusion.

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PURIFIED PLASMA KALLIKREIN INDUCE SEVERE SYSTEMIC VASODILATION BUT IS WITHOUT EFFECT ON THE PULMONARY CIRCULATION. F. Naess*, O. Roeise*, J. O. Stadaas*, A. O. Aasen. Dept. of Surgery, Ullevaal Hospital, University of Oslo, Norway.

Signs of activation of the plasma kallikrein-kinin system have been found in septicemia, ARDS and after severe trauma as well as in experimental endotoxemia. The precise effect of activated kallikrein *in vivo* have not been extensively studied, however. The recent purification of plasma kallikrein free of contaminating endotoxin have made these studies possible. Purified plasma kallikrein in a dose of 0.3 U/kg was injected I.V. into pigs. This resulted in marked reductions in systemic blood pressure lasting for 2-5 min, and was accompanied by increases in

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cardiac output. The calculated systemic vascular resistance thus was reduced to 28 % of baseline values, and the left ventricular stroke work also declined after the injection. The changes were rapidly reversible. The injection did not affect the pulmonary arterial pressure, and the calculated pulmonary vascular resistance was only modestly reduced. The dose of 0.3 U/kg of plasma kallikrein was so small that no effect on plasma prekallikrein, kallikrein or kallikrein inhibition was detectable using chromogenic peptide substrate techniques. Activation of plasma kallikrein thus may be one possible basis for the induction of a "hyperdynamic", vasodilated circulation in septicemia.

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EFFECTS OF LEUKOCYTE AND ERYTHROCYTE SUSPENSIONS ON FLOW RESISTANCE IN THE ISOLATED, PERFUSED RAT LUNG

T. Wikström*, M Braide*, U Bagge*and B. Risberg.
Univ. Gothenburg, Department of Anatomy and Surgery, Östra sjukhuset, Göteborg, Sweden.

Leukocyte and erythrocyte suspensions were given as bolus injections during cell-free, constant pressure perfusion of isolated rat lungs. Ventilation was maintained by a constant volume respirator and weight was continuously monitored to ensure isogravimetric conditions. Flow resistance changes were computed from registrations of flow rate, arterial and venous pressures (cf Braide et al., Am J Physiol, 256: H1117-H1126, 1989). The preparation showed a continuous efflux of leukocytes which had been trapped in the pulmonary microcirculation before the start of cell-free perfusion. Erythrocytes infusions (3 ml, hematocrit: 30%) increased the efflux of leukocytes. A transient flow resistance peak was seen during the passage of an erythrocyte bolus through the vascular bed but resistance promptly returned to a level at, or below, the baseline value. Infusions of mixed cell suspensions (20 - 30 x 10^6 leukocytes in 30% hematocrit) caused a transient resistance peak and a sustaied resistance increase. The sustained resistance increase was smaller amd less stable than the corresponding increase, seen after infusions of comparable numbers of leukocyte alone (Braide et al., 1989).
In conclusion, the present data suggest that infusions of erythrocytes caused a re-distribution and an increased efflux of pooled leukocytes in the pulmonary microcirculation. When the erythrocytes were infused in mixture with leukocytes, they reduced the leukocyte effects on flow resistance. The present rheological effects of erythrocytes could,hypothetically, result from mechanical interactions with the leukocytes in the pulmonary microvessels.

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EXPRESSION OF A 72kDa HEAT SHOCK PROTEIN IN NEUTROPHILS OF CRITICALLY ILL PATIENTS.

I. Kindas-Mügge, A.F. Hammerle, I. Fröhlich, F. Trautinger, M. Micksche (Spon: G. Schlag)
Institue of Cancer Research and Sepsis Research Group, Dept. of Anesthesia and Intensive Care Medicine University of Vienna, Austria.

Polymorphnuclear neutrophils (PMN), through their ability to release oxygen free radicals and other tissue damaging molecules, have a major function in patients suffering from sepsis and multiple organ failure syndrome (MOFS). Stress proteins, also designated heat shock proteins (hsp), play a physiological role in cellular repair and protecting cells from subsequent trauma. The "hsp 70 kDa" family is among one of the most prominent classes of heat shock proteins. In this study we analysed the expression of the inducible hsp72 protein, one of the major members of this family and the respiratory burst activity (RBA)of PMN in critically ill patients. Increased levels of hsp72 were found intracellulary in PMN of 9 out of 18 patients.

In vitro induction of hsp expression, by incubation of PMN at 42° C , was associated with a decrease in RBA in patients as well as in healthy donors. However, increased levels of hsp72 in vivo were not necessarily correlated with an inhibition of RBA in PMN. A correlation between hsp72 expression in vivo and clinical outcome, body temperature, respiratory parameters and liver function probes could so far not be detected. However, as hsps are representing an inducible repair system, further detailed study might detect clinical relevance of hsp in critically ill patients.

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ACTIVATED KUPFFER CELLS DURING SEPSIS - ADVANTAGEOUS OR JEOPARDIZING?

X.J.Meng, P.A.Qiu, Inst Basic Med Sci, General Hosp PLA, Beijing, China

Our previous work has shown that liver is the most susceptible organ during sepsis.This is to study the effects of Kupffer cell(KC) blockade on hepatocytes on the assumption that activated KCs may produce pathologic changes in sepsis. Healthy Wistar rats weighing 160-210g were used. Sepsis were produced by cecum ligation and perforation(CLP).Methyl palmitate 100mg/100g iv 24 hr prior to CLP reduced the phagocytic index from 0.049 to 0.015. At the end of 15 hr after CLP, animals were sacrificed and liver removed for assay. Adenosine triphosphate(ATP) measured by bioluminen-

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scence ; glutathione (GSH) measured spectrometrically; acetoacetate(AcAc) and 3-hydroxybutyrate(3-OHHD) enzymatically and prostaglandin(PGE_2) by RIA.

Results

	Liver			
	PGE_2 pg/mg	ATP umoles/g	GSH ug/g	AcAc/3-OHHD
Control(6)	906.8± 85.3	2.3849±0.16	21.86±1.38	2.78±0.48
Sham OP(6)	1071.0± 72.4	2.3146±0.13	22.21±0.71	2.61±0.67
Sepsis(6)	1543.0± 55.8	1.6909±0.07	16.00±0.97	0.26±0.06
Sepsis-KC Block(6)	732.7±134.2*	2.3332±0.13*	22.23±1.13*	0.68±0.14**

*p/ 0.01; **p/ 0.05 as compared to sepsis group.

Conclusion;- Although activated KCs may be advantageous in the initial elimination of pathogens, they accomplish this end at the potential cost of considerable tissue injury. This may explain the susceptibility of liver during sepsis and the sequential failure of multiple organs.

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POSSIBLE DISSOCIATION OF F_1-ATPASE FROM F_0 INDUCED BY HEMORRHAGE IN JAUNDICED LIVER. S. Iwata*, A. Tanaka*, Y. Takada*, H. Higashiyama*, Y. Shimahara* and K. Ozawa* (Spon: H. Hirasawa) Department of Surgery, Faculty of Medicine, Kyoto University, Kyoto, 606 Japan.

We have reported that liver mitochondria from jaundiced rats after 1 hour hypotension demonstrated a marked decrease in ATPase activity without remarkable changes in either oxidative activity or membrane potential of liver mitochondria. To clarify the cause for the reduction of this enzyme, oligomycin sensitivity and the ATPase activity of supernatants obtained from submitochondrial particles were examined. Polyacrylamide gel electophoresis (PAGE) analysis of catalytic part (F_1) of ATPase complex was performed. Hemorrhagic shock was induced according to Wiggers' model (mean arterial blood pressure: 40mmHg) in jaundiced rats produced by common bile duct ligation and sham operated rats as controls. All of the jaundiced rats after over 1 hour hypotension died although the shed blood was reinfused.

Fraction	ESMP		Supernatant from ESMP	
	Control	Jaundiced	Control	Jaundiced
ATPase activity (%)	100±6	52±4*	4±1	34±2*
Oligomycin sensitivity (%)	88±1	67±1*	78±3	16±1*

ESMP: EDTA submitochondrial particles, * P<0.001 compared with control.

SDS-PAGE analysis revealed that there was no alteration in the amount of F_1. These results showed the possibility that the function of oligomycin sensitivity conferring protein in binding F_1 to the membrane integral sector (F_0) of ATPase is compromised. This enzyme may play a key role in energy restoration in recovery from shock.

S11: Biological Monitoring

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BIOLOGICAL MONITORING. Richard G. Fiddian-Green, University of Massachusetts Medical Center, Worcester, MA USA 01655

The primary objective in monitoring patients is, as the Latin origin of the word implies, to warn clinicians of impending complications in time to implement therapeutic changes that may avert the complications. To determine which of the measurements being used in our ICU was an effective monitor in this sense a logistic regression analysis was performed on a database of all measurements recorded prospectively on the day of surgery in 85 patients having elective cardiac operations, all of whom had a pulmonary artery catheter. The only measurements found to be of stand-alone predictive value for complications within 72 hours of surgery were the systolic blood pressure, the duration of hypotension, the arterial pH and the duration of an arterial acidosis, the intramucosal pH in the stomach and the duration of the intramucosal pH in the stomach. The best stand-alone predictor was the intramucosal pH in the stomach and the best predictive model for impending complications derived from the measurements of systolic blood pressure and the intramucosal pH in the stomach. Neither the measurements made with a pulmonary artery catheter nor measurements of mean arterial pressure were of any predictive value for impending complications. Oliguria was of no predictive value and developed in only 1 of the 85 patients. In a further analysis of 323 measurements recorded in 60 ICU patients hypotension was found in 0.6% of measurements in 3.3% of the patients; an arterial acidosis in 3.1% of measurements in 8.3% of patients; and an intramucosal acidosis in the stomach in 71% of measurements in 72% of the patients. Hypotension and an arterial acidosis were usually preterminal and occasionally admission findings. Intramucosal acidosis developed intermittently throughout the stay in the ICU and in patients who died preceded the development of hypotension and arterial acidosis by many hours or even days. It is concluded that biological monitoring is most effectively performed by measuring the intramucosal pH in the stomach, the blood pressure and the arterial blood gases.

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CONTINUOUS MEASUREMENT OF PERIPHERAL TISSUE OXYGENATION IN PATIENTS WITH SEPSIS: EFFECT OF SYSTEMIC PSEUDOMONAS-IMMUNOGLOBULIN TREATMENT ON MEAN SKELTAL MUSCLE PO_2

P. Boekstegers*, St. Weidenhöfer*, K. Werdan
Dept. of Internal Medicine I, Klinikum Großhadern, University of Munich, FRG

In contrast to cardiogenic shock mean skeletal muscle pO_2 is high in patients with sepsis and multiple organ failure. The aim of the study was to find out:

1. the time course of mean muscle pO_2 elevation in septic patients
2. the effect hereon of supplemental sepsis treatment by pseudomonas-immunoglobolin

Therefore, in septic patients (n=5) polarographic pO_2-catheters were inserted within biceps muscle for up to 18 days in order to get continuous recording of peripheral tissue oxygenation. Continuous measurement by means of flexible and smooth pO_2-catheters was controlled by intermittent determination of the pO_2-distribution using polarographic needle electrodes in the vicinity of the pO_2-catheter. Mean skeletal muscle pO_2 obtained by these two methods was linearly correlated (r=0,89;n=30). Thus, it was possible to monitor reliably and continuously mean skeletal muscle pO_2 in intensive care patients (calibration of the pO_2-catheter needed every 4th day). In all five septic patients mean muscle pO_2 was higher than 40 mmHg for at least one day (up to 8 days). Highest values of mean muscle pO_2 were obtained in severest stage of sepsis with multiple organ failure (defined by APACHE II score). In two patients treatment by pseudomonas-immunoglobulin (Psomaglobin) was followed by a decrease of mean muscle pO_2 by 10-25 mmHg lasting 1-2 days, whereas systemic vascular resistance was unchanged. Repetitive administration of Psomaglobin (4-8 ml/kg/day, 40-50g) resulted in a repetitive decrease of mean muscle pO_2. In contrast albumin infusion (40-50g) did not change mean muscle pO_2. In the patients who overcame sepsis, mean muscle pO_2 returned to the physiological range (25-35 mmHg) in contrast to patients who died from septic shock. Conlusions: Our data indicate that continous and reliable monitoring of mean muscle pO_2 by means of pO_2-catheters is possible and provides useful information whether supplemental sepsis treatment has an effect on peripheral skeletal muscle oxygenation.

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CELLULAR INJURY SCORE AS AN INDEX OF THE SEVERITY OF PATIENTS WITH MULTIPLE ORGAN FAILURE

H. Hirasawa, T. Sugai*, Y. Ohtake, S. Oda*, H. Shiga*, K. Matsuda* and N. Kitamura*
Department of Emergency and Critical Care Medicine, Chiba University School of Medicine, Chiba, Japan 280

It has been claimed that multiple organ failure (MOF) is the summation of the cellular injury in vital organs. Therefore the severity of the patients with MOF can be accurately evaluated through the parameters which can express the severity of cellular injury. The present study was undertaken to investigate whether cellular injury score (CIS), which is derived from three different cellular metabolic sequences, arterial ketone body ratio (AKBR), serum osmolality gap and blood lactate, would be a good index to evaluate the severity of the patients with MOF. CIS was calculated daily on 106 MOF patients who were treated in our institution between 1985 and 1990. The correlations between the outcome of the patients with MOF and CIS, and the number of failing organs and CIS were studied. Also APACHE II was calculated on those patients and the accuracy of the outcome prediction of the MOF patients was compared between CIS and APACHE II. CIS significantly correlated to the number of the failing organs and to the outcome of MOF patients. CIS of survived patients at the onset of MOF was 2.2 ± 0.9 and that of non-survived patients was 4.1 ± 1.2 ($p < 0.05$), respectively. There were few survivors among MOF patients whose CIS was above 6.0. Furthermore CIS could more accurately predict the outcome of the MOF patients compared to APACHE II. Those results indicate that CIS is a useful and excellent index of the severity of the patients with MOF and suggest that the initiation of the therapeutic approaches against MOF among critically ill patients may be decided through the changes in CIS values.

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CLINICAL RELEVANCE OF MONITORING PLASMA LEVELS OF PROTEINASES, PROTEINASE INHIBITORS, AND CYTOKINES IN THE COURSE OF MULTIPLE ORGAN FAILURE

M. Jochum[1], D. Inthorn[3], Th. Jaka[4], W. Machleidt[2], Ch. Waydhas[3], H. Redl[5], H. Fritz[1] Department Clinical Biochemistry[1], Physiological Chemistry[2], and Surgical Clinics[3] of the University of Munich; Surgical Clinic[4] of the University Essen, Germany. L. Boltzmann Institute for Experimental and Clinical Traumatology[5], Vienna, Austria.

Repair and healing or perpetuation of inflammation in response to inflammatory noxae (e.g. multiple trauma, infection) depend on complex interactions of humoral and cellular defence mechanisms. Out of the various inflammatory parameters investigated hitherto, proteolytic enzymes, both of the plasma cascade systems (plasma kallikrein, thrombin, plasmin, complement esterases, etc.) and of lysosomal or granular origin (PMN elastase, macrophage cathepsin B, mast cell tryptase) have been shown to be potent effectors of destructive processes contributing to the occurrence of multiple organ failure (MOF) in severe posttraumatic and postoperative courses. Such proteolysis-induced pathomechanisms are greatly enhanced by the concurrently arising (local) imbalance between proteinases and their inhibitory regulators (e.g. α_1-proteinase inhibitor, α_2-macroglobulin, antithrombin III, PAI-1, C1-inactivator). In this respect, cytokines such as TNF or NAP (IL-8) seem to be relevant candidates for sequestration and activation of PMN granulocytes thereby increasing significantly the proteinase burden at the inflammatory focus. In several clinical studies on patients suffering from multiple trauma and/or septicemia we could demonstrate that measurement of cell-derived proteinases (PMN elastase, cathepsin B) and factors of the blood cascade systems (prothrombin, antithrombin III, protein C, C1- inactivator, PAI-1, etc.) in consecutive plasma samples turned out to be a helpful tool for early diagnosis and prognosis of severe multiple organ failure. In contrast, plasma levels of TNF and NAP monitored similarly showed only minor diagnostic significance. Yet, quantification of these cytokines in local body fluids (e.g. bronchoalveolar lavage fluids) clearly indicate their pathogenetic relevance for the development of organ failure.

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PROGNOSTIC VALUE OF COMPLEMENT ACTIVATION PRODUCTS IN POLYTRAUMA PATIENTS. G.Zilow, R.Burger, H.Redl and multicenter trauma study Vienna. Inst. of Immunology, Univ. of Heidelberg, 6900 Heidelberg; Robert-Koch-Inst BGA, 1000 Berlin, FRG; Ludwig Boltzmann Inst. for Experimental Traumatology, Vienna, Austria.

The adult respiratory distress syndrome (ARDS) is a common complication of traumatic and septic shock. Complement (C) induced activation of polymorphonuclear leucocytes (PMNs) and pulmonary leucostasis have been considered as important pathogenic factors in the development of ARDS. The C-activation products C3a and C5a are known to cause aggregation and adherence of neutrophils to lung vascular endothelium and release of destructive enzymes and oxygen radicals. The importance of C-activation in the early phase after polytrauma should be reflected by high plasma levels of split products. In a prospective study of polytrauma patients C-parameters and the C-activation products C3a-desArg, C3b(Bb)P and C1rsC1Inhibitor were determined in daily plasma samples over a period of 14 days with sample intervals of 6 hours during the first 48 hours. C-activation leading to a decrease of the C-proteins C3, C4, C5 and C1Inhibitor was observed in all patients. The inhibitors factor H, I and carboxypeptidase N did not differ in patients with or without ARDS. In contrast, significantly elevated C3a-desArg levels in the first 6 hours after injury were found in patients who later developed ARDS. A more sensitive indicator than C3a-desArg alone was the calculated C3a-desArg/C3 ratio. Since the prognostic value of C3a-desArg and the C3a-desArg/C3 ratio may be limited by their transient early rise short intervals in blood sampling should be necessary. In a second prospective study of trauma patients with plasma samples collected daily significantly elevated C3a-desArg levels in the first few days were found in patients with multisystem organ failure. C3a-desArg levels were significantly higher in non-survivors than in patients who survived. A new and simplified ELISA system using a monoclonal antibody reacting with a neoepitope of C3a-desArg, which is not present on the uncleaved C3 molecule facilitates specific and highly sensitive C3a-desArg measurement in patients plasma.

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Biochemical mediators in monitoring multiple injured patients

D.Nast-Kolb, Ch.Waydhas, M.Jochum, K.H.Duswald, L.Schweiberer

100 patients (mean ISS 37 pts.) were prospectively studied for a period of at least 14 days following trauma. 16 patients died with multi organ failure (between days 4 and 28), 47 patients survived with and 37 without organ failure. 27 showed signs of bacterial sepsis, 8 of them died, 2 had sepsis without organ failure. The rate of pneumonia was 46%.

For a large number of humoral and cellular mediators of inflammation no difference in their mean values was found between patients with different failing organs or patients with or without bacterial sepsis or pneumonia, respectively. However, some parameters, such as PMN-Elastase, Cathepsin B, Neopterin, C-Reactive Protein and AT III showed a highly significant correlation with the severity of organ failure (MOF-score <5 vs. ≥5). These biochemical mediators and indicators allowed to significantly distinguish between later death and survival as well as between survival with or without organ failure. It is thus possible to predict organ failure (Cathepsin B, PMN-Elastase, AT III) and death (Lactate, PMN-Elastase, C-Reactive Protein, Neopterin). Above that, these parameters are of diagnostic help to evaluate the course of trauma patients. Continously elevated plasma levels mean a high risk. In such situations large elective surgery with its additional trauma and further release of mediators may be contra-indicated.

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SIGNIFICANCE OF PLASMA ENDOTOXIN MEASUREMENTS. S.J.H. van Deventer, C. Wortel, A. Sturk, J.W. ten Cate. Center for Hemostasis, Thrombosis, Atherosclerosis and Inflammation Research, Academic Medical Center, Amsterdam, The Netherlands

We have previously shown that endotoxemia (endotoxin levels >5ng/L), as detected by the chromogenic Limulus assay (detection limit in blood: 3ng/L), accurately predicts the development of septicemia in febrile patients (sens.79%, spec. 96%). We have now determined the significance of endotoxemia in patients with severe Gram-negative septicemia (mean APACHE II score: 27) that were enrolled in a clinical study to determine the efficacy of HA-1A, an anti-endotoxin human monoclonal antibody. Eighty-two consecutive patients were enrolled in the study. Gram-negative bacteremia was present in 32, endotoxemia in 27. Endotoxemia and bacteremia showed a poor, albeit significant correlation ($R = 0.4$ $p = 0.001$). HA-1A treatment resulted in a significant reduction of TNF levels and mortality. The presence of endotoxemia showed no correlation with 28 day mortality, but predicted the efficacy of antiendotoxin treatment: in HA-1A treated endotoxemiac patients mortality reduced from 73% to 31% (change: -58%), in non-endotoxemic patients from 57% to 36% (change: -37%, $p<0.05$). In conclusion, endotoxemia predicts 1) the development of septicemia in febrile patients and 2) the efficacy of anti-endotoxin treatment in septic patients.

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DETERMINATION OF ENDOTOXINS,TNFa AND IL-6 IN INTRAABDOMINAL SEPSIS -THE PROGNOSTIC VALUE IN THE PREDICTION OF SURVIVAL AND PULMONARY FAILURE. R. Függer, F. Schulz, M. Rogy, M. Prager, E. Kyral, G. Hamilton, A. Fritsch
I. Chir. Universitätsklinik, University of Vienna, Alser Straße 4, 1090 Wien, Austria.

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Endotoxins and the cytokines TNFa and IL- 6 are known to play an important role in the pathophysiology of septic shock. The aim of this study was to search for a correlation between the plasma levels of these mediators and survival in severe intraabdominal infection. Additionally a possible correlation between endotoxins, TNFa and IL-6 and the establishment of pulmonary failure (defined as need for respirator therapy) was studied.
Methods: Since November 1989 18 patients (diffuse peritonitis 9, intraabdominal abscess 2,infected pancreatic necrosis 7) entered this prospective study. Five of them died due to multiple septic organ failure. Heparinized blood samples were colleted three times a day (8 AM, noon, 4 PM), beginning at the time of diagnosis until discharge from the intensive care unit or death. Endotoxemia was determined with a chromogenic modification of the limulus amebocyte lysate test, TNFa with an immunoradiometric assay (Medgenix, Brusssels, Belgium) and IL-6 with an ELISA kit (Quantikine IL-6, R+D Systems, Minneapolis, USA).
Results: The median plasma levels of survivors compared with non-surviors for endotoxin (12 vs 9EU/ml, p=0.18 Wilcoxon) and TNFa (12 vs 21 pg/ml, p=0.44) were not significantly different. However, median IL-6 levels were markedly elevated in non-survivors (171 pg/ml) than in survivors (82 pg/ml, p=0.02). Furthermore there was a correlation between pulmonary failure and IL-6. Median values of IL-6 on days with pulmonary failure (159 pg/ml) were significantly higher than those on days with need for respirator therapy (70 pg/ml, p=0.0098). Endotoxemia (11 vs. 13 EU/ml, p =0.26) and TNFa (12 vs 15 pg/ml, p=0.42) were not significantly different in patients with an without pulmonary failure.
Conclusion: In severe intraabdominal infection IL-6 was found to be of significance with respect to survival and respiratory failure, while endotoxin and TNFa failed to differentiate in these terms.

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PMN - RESPIRATORY BURST ACTIVITY AND TNF - ALPHA (TNF-a) IN CRITICALLY ILL PATIENTS.
A.F. Hammerle, P. Germann, W. Macheiner, N. Mayer, M. Micksche, F. Trautinger, H. Steltzer, C. Weinstabl, (Spon: H. Redl)

Oxygen free radicals liberated by neutrophil granulocytes and neutrophil stimulating mediators such as Tumor Necrosis Factor-a (TNF-a) are thought to be involved in the pathophysiology of sepsis and multiple organ failure syndrome (MOFS). In critically ill patients at high risk for the developement of septic syndrome peripheral blood neutrophils were assayed for O_2^- and H_2O_2 production after stimulation with Phorbol Myristate Acetate (PMA 40 nM). Serum TNF-a levels were determined by ELISA. 17 critically ill patients (12 males, 5 females, age 51±9 years, range 34-69 yr) were included in the study. Diagnosis of the patients were polytrauma, major surgery, cranial injury, pancreatitis, intoxication, burn injury and cirrhosis hepatis. At the time of admittance to the intensive care unit all of the patients were severely ill but did not show clinical manifestations of septic syndrome. During follow up 7 out of 17 patients developed sepsis. Mean time of follow up was 12 days (range 4-41). At the time of admittance, when no symptoms of sepsis were detectable by routine measures, significant higher TNF-a serum levels (56,3±11,4 pg/ml; mean±SE) were found in the group of patients finally developing sepsis compared to the nonseptic group and to the healthy donors (p=0.0001). Patients not developing septic syndrome had lower TNF-a serum levels, that did not significantly differ from TNF-a values in healthy volunteers (10,7±5,8pg/ml, 5,9±4,2 pg/ml; mean±SE, respectively). During further follow up the elevated TNF-a levels in septic group remained high whereas in the control group values never exceeded the normal range. Simultaneously with TNF-a determination respiratory burst activity of neutrophils was investigated in patients and healthy volunteers. Again, at the time of admittance spontaneous as well as PMA induced O_2^- release (26.5±4,3 nmol/h/10^6 cells, 51,6±7,3 nmol/h/10^6 cells; mean±SD, respectively) was significantly elevated in the group of patients finally developing septic syndrome compared to nonseptic patients and healthy donors. These results demonstrate that even when no clinical signs of sepsis were present in the patients investigated elevated values for both, serum TNF-a and neutrophil respiratory burst activity could be detected. These results give further evidence, that neutrophil granulocytes and their products might play a significant role in the symptomatology of sepsis.

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PMN-ELASTASE (El) AND NEOPTERINE (Ne) PLASMA LEVELS IN SEPTIC (SeP) AND IN UNCOMPLICATED (NSeP) PATIENTS AFTER CARDIAC SURGERY (CS).
G. Pilz, S. Kääb, E. Kreuzer*, A. Heubner** and K. Werdan, Depts. of Medicine I and of *Cardiac Surgery, Grosshadern Univ. of Munich Hospital, **E. Merck, Darmstadt, Germany.
We investigated plasma El and Ne with regard to the early evaluation of post-operative (pop) sepsis in patients (P) after extracorporeal circulation (ECC), a potential cause of unspecific El and/or Ne increase. Daily measurements (El: IMAC-El Merck; Ne: RIA IMMUtest Henning, Berlin, Germany) were done in 110 P consecutively admitted to the ICU after open-heart CS requiring ECC, excluding 1 P on chronic hemodialysis (unspecific high El and Ne already at baseline). In the remainder 109 P, sepsis (defined as Elebute sepsis score of ≥12 on ≥2 days) occurred in 16 P (SeP) and was associated with a significantly worse prognosis (mortality 69% vs 1%, p<0.0001) compared to non-SeP (NSeP).
Results (mean±SEM. p-values: Wilcoxon or Mann-Whitney test, resp.): While in both NSeP and SeP El displayed a highly significant (p<0.0005) peak on the evening of the operation day ("0") with a prompt fall in the consecutive days, no such peak was noted for Ne, which in turn showed a subsequent upward trend in both groups. Most important, however, both parameters were nonetheless significantly higher (p<0.0005) in SeP vs NSeP already on pop day 0, as well as on days 1 and 2. Furthermore, El and Ne discriminated (p<0.05) between surviving (su) and non-su SeP (El: pop day 1, Ne: pop days 0-2).

Patients	n	day:	-1	0	1	2	day:	-1	0	1	2
NSeP all	93	El(µg/l):	29±1	262±15	108±6	81±3	Ne(nmol/l):	9±1	12±2	24±3	35±4
SeP all	16	El(µg/l):	47±7	415±34	230±32	170±24	Ne(nmol/l):	27±6	34±7	64±14	66±11
SeP su	5	El(µg/l):	33±5	432±70	139±13	133±13	Ne(nmol/l):	11±2	10±1	27±4	35±7
SeP non-su	11	El(µg/l):	54±9	407±40	272±42	190±36	Ne(nmol/l):	34±7	44±9	81±19	81±14

Conclusion: Despite ECC, El and Ne might be useful to early differentiate between CS patients at either high or low risk for developing post-operative septic complications.

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CIRCULATING INTERLEUKIN (IL)-6, BUT NOT TNFα, IS STRONGLY ASSOCIATED WITH OUTCOME IN SEPTIC SHOCK PATIENTS. H.G. Kress, R. Götz* and E. Heidbreder*.
Depts. of Anesthesiology and *Nephrology, University Hospital, D-8700 Würzburg, Germany.

Although cytokines play a central pathogenetic role in inflammation and sepsis, no strong correlation could be demonstrated between circulating cytokines and sepsis mortality. In our institutionally approved, prospective study it was attempted to correlate the plasma levels of cytokines (IL-1, IL-6, TNFα) and soluble IL-2 receptors (IL-2R) with the clinical outcome in patients suffering from developing septic shock. **Methods:** A total of 24 consecutive patients entered the study not later than 24 h after onset of septic shock symptoms (leukocytosis/leukopenia, fever, hemodynamic disturbances, respiratory and renal failure). Plasma samples from central venous blood were collected at defined time intervals (-80°C). Cytokines were analyzed using commercial test kits for IL-2R (*Cellfree*®, *T Cell Sciences*), human IL-6 (*Quantikine*®, R&D Systems), human TNFα (IRMA, Medgenix), and IL-1(RIA, Medgenix). **Results:** The observed mortality rate was 46% (11/24). In the first 3 days after allocation to the study group, plasma levels of IL-2R, IL-6 and TNFα were elevated similarly in every individual. At this early stage, the magnitude of an individual's plasma level did not reflect his clinical outcome. In the later course, however, a striking correlation was found between the profiles of IL-6 production and mortality from septic shock ($p<0.01$). IL-6 progressively declined in survivors, whereas it significantly increased again in the plasma of every non-survivor many days before death. No such correlation could be detected for TNFα or other cytokines. **Conclusion:** In a well-defined population of septic shock patients neither TNFα nor IL-1 and IL-2R correlate with clinical outcome at any time interval. In contrast, the plasma profiles of IL-6 provide a reliable basis for prediction of a fatal outcome. However, whether and how IL-6 production may be related to the development of fatal organ failure remains unclear.

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A PREDICTIVE MODEL FOR THE CLEARANCE OF CIRCULATING PHOSPHOLIPASE A_2 DURING SEPTIC SHOCK IN MAN

Peter Vadas *, Waldemar Pruzanski *, Vern Farewell * (Spon: J. Filkins)
Wellesley Hospital, Toronto, Ontario, Canada M4Y 1J3

A soluble phospholipase A2 (PLA2) is released into the systemic circulation in response to the presence of bacteria or bacterial products. In retrospective and prospective studies of septic shock in man, circulating PLA2 correlated with the severity and duration of circulatory collapse. Infusion of purified exogenous PLA2 produced hypotension in experimental animals. Thus, circulating PLA2 has been recognized as a mediator of cardiovascular collapse in septic shock. Proximal mediators of endotoxemia, including TNF and IL-1, induce PLA2 synthesis and secretion in vitro and in vivo, but the factors regulating PLA2 elimination are unknown. Similarly, the kinetics of PLA2 clearance during recovery from septic shock have not been determined. The kinetics of PLA2 clearance in 15 patients with septic shock were analyzed from the point of maximal hypotension until recovery. An autoregressive mathematical model was developed to describe the rate of PLA2 clearance during the recovery phase of septic shock. This model accounted for almost 90% of the variability seen in the data. The calculated circulating half-life of soluble PLA2 in septic shock in man was 32 hr. Since elevation of serum PLA2 activity is closely associated with bacteremia or endotoxemia, a significant deviation from predicted PLA2 values may denote impending relapse.

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BIOCHEMICAL MONITORING OF POSTTRAUMATIC LUNG FAILURE: INVESTIGATING ALVEOLAR PERMEABILITY FOR SERUM PROTEINS (ESP. ALBUMIN) BY MEANS OF SERIAL BRONCHOALVEOLAR LAVAGE IN TRAUMA PATIENTS

U. Obertacke, Th. Joka, M. Jochum[1] and K.P. SCHMIT-NEUERBURG
Dep.of Traumatology Univ. Essen, [1]Dep. Clin. Biochem., Univ. Munich FR Germany

In trauma patients (ISS > 40 points) we performed bronchoalveolar lavage (BAL) as well as the first clinical measurements within the first 6 hours after admission to hospital. There after BAL was carried out once per day. BAL protein concentrations were converted to epithelial lining fluid (ELF) according to REINHARD (JApplPhysiol60(1986)532). The alveolar capillary permeability (ACPerm) was described by the quotient of the protein concentrations [ELF/Plasma]. Progressive lung failure (ARDS) was defined using the criteria given by MURRAY (ARRD138(1988)720). The study period was 14 days. RESULTS: Data from 12 patients were evaluated. 5 patients developed a progressive lung failure ("+ARDS"), in 7 patients no pulmonary failure was observed ("-ARDS"). An increased ACPerm for albumin was detectable for both +ARDS- and ARDS-patients after the 6th hour, reaching a maximum around the 24th hour. +ARDS patients showed a significant higher ACPerm. Further, beginning from the 48th hour to 4th day, the ACPerm-value for -ARDS-patients again dropped to a physiologic range. The degree of the increased ACPerm depends on the size of the molecules forming the marker protein (i.e. albumin or alpha 2 MG), but is independent of pulmonary hemodynamics. It is of value for the prediction of ARDS within 24 hours, and for monitoring of pulmonary damage in posttraumatic course. The early increased ACPerm for proteins may be one effect of an early (reversible) "organ in shock syndrome" in trauma patients. Meanwhile these data have been confirmed in a further prospective study.

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EVIDENCE FOR THE ROLE OF LYSOSOMAL CYSTEINE PROTEINASES AS MEDIATORS OF INFLAMMATION IN ENDOTOXIN SHOCK, POLYTRAUMA AND ORGAN FAILURE.

W. Machleidt, I. Assfalg-Machleidt, M. Jochum, D. Nast-Kolb, M. Siebeck, A. Billing, H. Hoffmann, Th. Joka and H. Riess (Spon: H. Redl).
Inst. Physiolog. Chem. Univ. Munich, Goethestr. 33, D-8000 München 2; Dpt. Clin. Chem. and Clin. Biochem., Surg. Clinic City Univ. Munich;, Surg. Clinic City Univ. Munich; Surg. Clinic Großhadern Univ. Munich; Surg. Clinic Univ. D-4300 Essen; Dpt. Int. Med. Univ. Hospital Rudolf Virchow, D-1000 Berlin.

The cysteine proteinases cathepsin B, H, L and S are most abundant in lysosomes of monocytes, macrophages and other cells of the reticuloendothelial system. Cathepsin B is loosely bound to its protein inhibitors (kininogens, cystatin C) and can be measured as active enzyme after dissociation. Elevated cathepsin B activity and reduced inhibitory capacity for cysteine proteinases were found in blood plasma of polytraumatized and septic patients as well as in pigs subjected to experimental endotoxin shock. High cathepsin B activity soon after the traumatic event proved to be a sensitive and specific parameter for the prediction of subsequent organ failure. In patients with lung failure (ARDS), part of the proteolytic activity of bronchoalveolar lavage fluid was due to cysteine proteinases not compensated by sufficient levels of inhibitors. Extremely high amounts of cathepsin B were released during reperfusion of human liver transplants. In vitro, cysteine proteinases are able to proteolyse and inactivate functionally important proteins (matrix proteins, immunoglobulins, proteinase inhibitors) and to generate biologically active peptides. Our results suggest that lysosomal cysteine proteinases released from cells of the mononuclear phagocyte system may act as non-specific mediators of inflammation contributing to the development of organ failure.

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CHANGES IN SERUM CARBOXYPEPTIDASE R (CPR) LEVEL IN RATS IN ENDOTOXIC SHOCK OR WITH PERITONITIS.

T.Hayakawa, N.Shinagawa, H.Takeyama, K.Kato, M.Taniguchi, Y.Akamo, J.Yura and W.Campbell*, H.Okada*. First Department of Surgery, Department of Molecular Biology*, Nagoya City University Medical School, Nagoya, Japan

Changes in the serum level of a novel arginine carboxypeptidase (CPR) that is generated during blood coagulation were observed in animals submitted to experimental endotoxic shock or peritonitis. This enzyme, which is distinct from carboxypeptidase N (CPN), removes terminal arginine from small synthetic substrates and may function in vivo by removing the terminal arginine from inflammatory peptides such as C3a and C5a thereby regulating the severity of inflammation. Male Sprague-Dawley rats weighing about 200 grams were used to monitor CPR levels during response to endotoxin as well as to artificial peritonites. Endotoxic shock was induced by administering lipoplysaccharide (E.coli 026:B6) intravenously at a dose of 0.5, 2 or 4mg/Kg. Peritonitis was induced by intraperitoneal injection of E.coli, mucin and barium sulfate. Blood sampling was carried out for each group before and after injection. Carboxypeptidase activity towards the substrate hippuryl-L-arginine was determined by HPLC. Changes in CPR activity, as measured by the difference between serum and plasma carboxypeptidase levels, were similar for all the 3 endotoxin doses. For example, in the 2 mg/Kg group, it was 73.2(U/L, Mean) before endotoxin administration; post-administration it was 82.4 after 30 min, 115.5 after 1 hour, 29.8 after 6 hours and 56.6 after 12 hours. In the peritonitis group it was 95.8 before intraperitoneal injection; post-injection it increased to 212.1 after 3 hours, then decreased to 40.7 after 6 hours, and was 19.4 after 12 hours. From these results it can be seen that following a transient elebation, serum CPR levels decreased to a subnormal level. And when they fell to zero the animals did not survive. In a clinical setting, measuring serum CPR levels may be useful for assessing the severity of infection.

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STUDIES ON FIBRINOLYSIS AND COAGULATION AS PROPHYLACTIC MEASURES IN BONE-SURGERY INTERVENTIONS.

W. Heller, Z. Engel
University of Tübingen, Dept. of Cardiovascular Surgery, Calwerstr. 7, D-7400 Tübingen, FRG

The patients were divided into five groups. In group 1 the patients had to undergo a removal of metallic implants. The patients in group 2 showed only slight woundings, such as broken ankle joints. In group 3 seriously injured patients with polytraumata were to be found. In group 4 fractures in patients with general diseases had been operatively treated. In group 5 all the operated patients suffered from a malignant basic disease. Following proteins of the coagulation and fibrinolysis were determined from the first preoperative up to the eighth postoperative day: fibronectin, antithrombin III, plasminogen, α_1-antitrypsin, α_2-macroglobulin and C1-inactivator. Groups 4 and 5 showed reduced concentrations of fibronectin, antithrombin III and plasminogen. In these groups the preoperative values were already 25% lower than in groups 1 and 2, to which they were compared. Whereas in group 4 variations of at most ± 8% of the initial level could be established, the concentration of fibronectin, antithrombin III and plasminogen on the first postoperative day was in the tumor group respectively 50%, and for the last two parameters 65% of the standard limit of tolerance. The

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investigations revealed group 3 as a conditional endangered group: the preoperative values between 6 and 13% below the normal range, were reduced during the operation at about 25%. The concentrations of C1-inactivator and α_1-antitrypsin were typically high, up to gushed.

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DOWNREGULATION OF INTRAVASCULAR ALTERNATIV PATHWAY ACTIVATION IS INDICATED BY COMPLEXES OF COMPLEMENT FACTOR H WITH C3 BREAKDOWN PRODUCTS. H. Gold*, R. Deppisch* and E. W. Rauterberg*, (Spon: H.Redel) Inst.Immunology, Univ.Heidelberg, INF305, D-6900 Heidelberg, and German Diagnostic Clinic, Aukammallee 33, D-6200 Wiesbaden, Germany.

Contact of blood with "foreign surfaces" as during hemodialysis results in an activation of the alternative pathway of complement (APC). In the present study we developed a new ELISA for the measurement of complexes of factor H (FH) with C3 breakdown products (i.e. FHC3b, FHiC3b, FHC3d). The monoclonal antibody M45 to human FH which was recently produced in our laboratory served as scavanger. Plasma samples containing 10 mM EDTA were analysed at dilutions of 1:50 and 1:300. Binding FHC3b (or FHiC3b) complexes were detected by rabbit anti-C3c and FHC3d complexes by anti-C3d followed by mouse anti-rabbit IgG alkaline phosphatase conjugates (Dianova). With CVs between 2% and 7% the assay exhibited a high precision and reproducibility. Optimal measuring range was between OD values of 0.200 and 1.200, respectively. Yeast activated serum served as arbitrary standard. Concentration of FHC3b and FHC3d was measured in the blood lines (arterial inlets and venous outlets) of five patients each subjected to hemodialysis with cuprophan (CU), hemophan (HE) or polysulfon F6 (PS) membranes (identical geometry). Complexes of factor H with C3 breakdown products revealed a high stability at 37°C in vitro. In vivo, two phases could be discriminated in the generation of FHC3b and FHC3d (dialysator outlet): An early phase (10-20 min) with a peak generation was followed by slower but steady increase over the rest of the measuring period. Generation of FHC3d or FHC3b was independent from the formation of terminal complement complexes (TCC) in individual patients. Inter-patient variation of FH-complex formation was higher than with TCC. However, the three types of membranes could be ranked with respect to their FH-complex generation in the order CU > HE > PS. Our findings suggest (i) that FHC3b and FHC3d complexes are formed in vivo, (ii) that they exhibit a suprisingly high stability, and (iii) that their measurement allow to estimate the APC downregulatory activity in plasma. Since complement activation is suggested to contribute to the pathogenesis of shock, we conclude that the new test might be of high value to analyse the downregulative capacity during various types of the disease.

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MONITORING OF INFECTIOUS COMPLICATIONS BY ROUTINE DETERMINATION OF PMN-ELASTASE, NEOPTERIN AND TNF-ALPHA. P. Krafft, J. Winternitz, O.A. Wagner, G. Pöschl, C. Oismüller, P.M. Winter and A.E. Hammerle (Spon: G. Schlag), Dept. of Anesthesia and General Intensive Care, Sepsis Research Group - University of Vienna, A-1090 Vienna

Infectious complications, the leading cause of mortality in critically ill patients, frequently present difficult diagnostic problems. The aim of our study was to investigate the validity of routine serum level determinations of PMN-Elastase, Neopterin and TNF for the diagnosis, course and prognosis of localized and septic infections. **METHODS:** Serum levels of PMN-Elastase, Neopterin and TNF were measured daily in 68 critically ill patients. In 43 patients who developed infectious complications, 17 had localized infections (LI) and 26 were septic (S) according to clinical criteria. Data were analyzed using the SAS software package (SAS Institute, Cary, NC) and are expressed as mean±SEM. **RESULTS:** The mean serum levels of Neopterin were sign. higher in LI than in patients without infection (33±1.9 vs 14±0.5 nmol/l), but both groups exhibited normal TNF values (<15pg/ml). Elastase concentrations were not sign. higher in group LI as compared to patients with non-infectious trauma- or surgically induced Elastase elevation. Neopterin (24.8±4.3 vs 152.4±24.6nmol/l) and TNF (6.9±0.9 vs 77.8±22.2pg/ml) enable a sign. differentiation between a localized infection and a septicemia by the first day of infection, while PMN-Elastase (143.3±20.8 vs 244.3±32.2mcg/l) did not allow that differentiation before day 3. The sensitivity and specificity of a single serum level determination for the diagnosis of a septic syndrome were in Elastase (>140mcg/l): 65% and 77%, in Neopterin (>50nmol/l): 84% and 95% and in TNF (>30pg/ml): 54% and 99%. The mean TNF (118.1±37.6 vs 36.8±24pg/ml) and Elastase (268.1±43 vs 175.3±34.3mcg/l) values of septic non-survivors were sign. higher than for survivors already after the first day of sepsis, while Neopterin first differentiated septic non-survivors from survivors by day 4 (190.2±39.5 vs 104±21.7nmol/l). **CONCLUSION:** Neopterin is a reliable parameter for the diagnosis and course of localized infections, while the determination of PMN-Elastase is, at least in patients after trauma or major surgery, of limited value. The parameter with the highest diagnostic sensitivity for septicemia is Neopterin, while TNF exhibits the highest diagnostic specificity. TNF and Elastase serum levels best reflect the prognosis of a septicemic episode.

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PARALLEL INCREASE OF NEOPTERIN, PHOSPHOLIPASE A2 and C-REACTIVE PROTEIN AFTER TRAUMA. R. Tikku*, R. Nitz*, U. Sonnekalb*, I. Marzi, and V. Bühren. Dept. of Trauma Surgery, University of Saarland, D-6650 Homburg/S., FRG.

Phospholipase A2, a key enzyme for the generation of leukotrienes and prostanoids, and neopterin, an unspecific marker of macrophage activation may be involved in the inflammatory response following trauma. The aim of this study was to determine the correlation of phospholipase A2, neopterin, C-reactive protein and PMN-elastase during the first 14 days in polytraumatized patients. Therefore, these parameters were assessed daily in 12 patients with a mean AIS-ISS score of 32.8 ± 2.6 (± SE). Phospholipase A2 was measured by ^{14}C-oleate-labelled E. coli bioassay, neopterin by RIA (Immu-test, Henning, Berlin, FRG), C-reactive protein by laser-nephelometrie, and PMN-elastase by an immuno-assay (IMAC, Merck, Darmstadt, FRG). Phopholipase A2 and neopterin values rose parallel during the first five days after trauma: e.g., phospholipase A2 at day 1: 1840 ± 1600 U/ml, at day 7: 36000 ± 3835 U/ml; neopterin day 1: 1.74 ± 0.21 ng/ml, at day 7: 4.02 ± 0.77 ng/ml ($p < 0.05$). A similar increase was observed by C-reactive protein levels (day 1: 66.4 ± 8.8 mg/l, day 7: 136.8 ± 21.8 mg/l; $p < 0.05$). During the subsequent days, a slow decrease of these parameters was

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observed in the majority of the patients. In contrast, PMN-elastase decreased from 147.5 ± 19,8 µg/l at day one continuously (e.g., day 7: 86.4 ± 8.7 µg/l). In conclusion, the parallel rise of neopterin and phospolipase A2 is consistent with an activation of macrophages in the first days after trauma. The similar time scale of the acute phase response, reflected by C-reactive protein levels, may be due to mediators released from activated macrophages (e.g., Interleukin-6). On the other hand, the inverse time course of PMN-elastase may indicate an earlier participation of leukocytes after trauma.

CLINICAL IMPLICATION OF ENDOTOXIN, PMN-ELASTASE AND PHOSPHOLIPASE A_2-ACTIVITY IN PATIENTS WITH MULTIPLE INJURIES. **219**

W. Uhl, D. Berger, A. Deller*, M. Vogeser, M. Büchler
Departments of Surgery and *Anaesthesiology, University of Ulm, Steinhoevelstr. 9, D-7900 Ulm, Germany

This prospective study shows data on comparative measurements of endotoxin (E), phospholipase A_2-activity (PLA_2) and PMN-elastase (PMN-E) in multiple injuries (MI). **Pats. and Methods:** 17 pats (10 male, 7 female, average age 35.7, range 16-78 ys.) with MI were included in this study. The median "injury severity score" was 43 (range: 29-66 units, the mortality rate 29% (5/17). A chromogenic modification of the limulus-amebocyte-lysate-test was used for the determination of E (normal value: ≤ 0.015 EU/ml) and PLA_2-activity was measured radiochemically (n.v.: 0-2 U/l). PMN-E was determined by an antibody-assay (n.v.: 10-95 µg/l). During the first 10 days of intensive care treatm. following MI a daily serum-monitoring was done. **Results:** 75% of MI pats. had increased serum levels already on the day of hospitalisation (day 0: median 0.17; range 0.015-1.0 EU/ml; n=16). PLA_2-activity was increased in all pats., with maximum values on day 5 (day 0: median 4.2; range 0.03-28.4 U/l; n=17; day 5: median 5.6; range 1.2-121.6 U/l; n=12). An immunoabsorption study with a specific antibody against pancreatic PLA_2 showed that this PLA_2-activity did not stem from the pancreas. PMN-E was increased on all days (day 0: median 197.1; range 41.9-529.3 µg/l; n=16; day 5: median 105; range 32.7-334.5 µg/l; n=7; day 7:median 180.3; range 24.2-317 µg/l; n=7). In linear regression analyses correlations between E and PMN-E were found ($r=0.62$; $p<0.0004$; n=113), between PLA_2-activity and the pO_2/FiO_2 quotient ($r=-0.5$; $p<0.0004$; n=117) and between E and the pO_2/FiO_2 quotient ($r=-0.32$; $p < 0.0003$; n=118). **Conclusions:** The correlation with PMN-E is pleading for a granulocyte stimulation through endotoxin, independent of the amount of leukocytes. The same is true for PLA_2-activation. The significant correlation of E and PLA_2 with pulmonary insufficiency during treatment can be judged as an essential prognostic index.

4-Hydroxynonenal (HNE) and PMN Elastase in Surgical Trauma Patients during p.p. and p.s. Healing **220**

[1]Kukovetz E., [2]Hofer H.P., [3]Egger G., [2]Wildburger R., [1]Schaur R.J.
1. Institute of Biochemistry, 2. University Clinics of Surgery, Dept. of Traumatology, 3. Institute of Functional Pathology, University Graz

While the proteinase Elastase is an established marker in inflammatory diseases, the product of oxidative lipid metabolism of PMN, 4-hydroxynonenal (HNE) has not yet been studied so far in venous blood in patients with trauma. In this study both parameters were measured in surgical trauma patients with "per primam" and "per secundam" wound healing. HNE was determined as 2,4-dinitrophenylhydrazone by reversed phase HPLC after preseparation by extrelut extraction and TLC. Elastase was quantified as α_1-antiproteinase-inhibitorcomplex by an immunoassay.
In the group of patients with aseptic physiological wound healing (p.p.) significant positive correlations were found between the number of PMN on the one side and the HNE concentration as well as the concentration of Elastase inhibitorcomplex resp., on the other side. In trauma patients with bacterial wound infection (p.s.) a significant positive correlation between HNE and Elastase was established.
The differential behaviour of both markers of inflammation in the two groups of trauma patients can be explained on the basis of differences in phagocytic activity of PMN.

CERULOPLASMIN AND LIPID PEROXIDATION IN PATIENTS WITH ACUTE ORGAN SYSTEM FAILURE. **221**

R. Dauberschmidt, I. Schimke, I. Förster, Ch. Stumpe, M. Meyer
Friedrichshain Hosp., Berlin, and Humboldt Univ., Sch.Med., Berlin, Germany

The development of an acute organ system failure (OSF) is connected with a decrease in the ceruloplasmin activity (CPL). In the blood, about 31 % of the antioxidative capacity is determined by CPL. To test for a possible relationship between decrease of CPL activity and lipid peroxidation 28 patients were investigated. CPL activity, malondialdehyde concentration (MDA), PMN-elastase (elastase) and lactate was measured daily. The number of organs developing an OSF (OSF/d) was diagnosed according to a standardized procedure. CPL activity <60 U/l and MDA concentration >9.28 µmol/l were regarded as pathological. Patients were grouped as follows: I: pathological CPL and MDA (n = 6), II: pathological MDA and normal CPL (n = 5), III: pathological CPL and normal

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MDA (n = 7) and IV: normal CPL and MDA (n = 10). Results are summarized in the following (median values):

Group	OSF/d	CPL (U/l)	MDA (µmol/l)	Lactate (mmol/l)	Elastase (µg/l)
I	2.5**	0.93*	10.46*	2.30	944
II	1.6	2.13***	10.00*	1.70	765
III	2.3	1.25**	5.62	1.90	697
IV	1.8	1.87	5.80	2.30	816

In group I only there is a strong correlation between decrease of CPL and increase of MDA ($r = -0.63$; $P < 0.001$). In comparison with group IV, the most severe pathological changes are to be found in group I (*: $P < 0.001$; **: $P < 0.01$; ***: $P < 0.05$). In this group, too, significant correlations exist between elastase or lactate, respectively, and OSF/d ($r = 0.63$ or 0.77; $P < 0.001$). Conclusion: In 46 % of the patients with low CPL activity the impairment of the antioxidative capacity is connected with an increase in lipid peroxidation.

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A NEW ENDOTOXIN AND CYTOKINE BLOOD SAMPLING AND STORAGE TUBE: NO MANIPULATION, NO CONTAMINATION, AND EASY HANDLING. G. Leichtfried, S. Bahrami, H. Redl and G. Schlag. Ludwig Boltzmann Inst. Exp. Clin. Traumatol., Vienna, Austria.

Despite the fact that highly sensitive assays are widely used for endotoxin and/or cytokine measurements in plasma samples of patients, the results are controversial. To prevent possible sample contamination following blood collection and storage, we have attempted to develop special collection and storage tubes, which are sterile and endotoxin-free and at the same time minimise handling throughout the sample preparation for the assays.

Methods: In co-operation with Greiner-Austria and Kabi Diagnostica we have developed special blood collection tubes prepared with endotoxin-free a) heparin (Immuno-Austria) and b) a silicone gel for cell/plasma separation. Commercially available standard tubes obtained from Becton-Dickinson (Vacutainer, 10 ml) were used for comparison.
The following tests were performed: Endotoxin contamination and recovery, and TNF production in blood samples following 2 hrs of incubation at 37°C.

Results: Standard blood collection tubes were found to contain about 400 pg LPS/tube as compared to <=4 pg LPS/tube in the special tubes. Endotoxin recovery was not influenced by the gel. Cytokine analysis revealed that plasma from standard blood collection tubes had higher TNF levels than from endotoxin-free tubes.

Conclusion: With the use of special blood collection tubes false positive results due to contamination during blood sampling and storage may be precluded.

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USEFULNESS OF ENDOTOXIN DETERMINATION IN THE MANAGEMENT OF INFECTIONS AFTER GASTROINTESTINAL OPERATION. Y.Yaegashi,[1,2] M.Watanabe,[1] K.Saito,[1] K.Inada,[2] M.Yoshida.[2] [1] Dept. of Surgery I, [2] Dept. of Bacteriology. Iwate Medical Univ., 19-1 Uchimaru, Morioka 020, Japan.
This study included 190 patients who were suspected of having infection following gastrointestinal operation. After the new PCA plasma pretreatment, endotoxin was determined by Endospecy assay. The normal level of endotoxin is less than 9.8 pg/ml. Twenty-five endotoxin positive patients received low-dose intramusclar injection of Polymixin B (12,500 U X 4 doses/day), and the effect of this therapy was assessed. Of 190 patients, 38 were diagnosed as having endotoxemia. The underlying disease was esophageal varices in 11 patients, colorectal cancer in 8, disease of liver, gallbladder or pancreas in 5, gastric cancer in 4, and other disease in 4. Complication by peritonitis was observed in 12 patients (33.3%). Sixteen patients (50.0%) died. After intramusclar Polymixin B therapy, plasma endotoxin level became negative in all patients within two days. Endotoxin level before treatment did not differ between survivors and non-survivors. When the course of endotoxemia was compared between survivors and non-survivors, non-survivors tended to show recurrece of endotoxemia, while in survivors, the conversion to endotoxin negative level reflected changes in their pathophysiological condition and was not followed by reccurrence of endotoxemia. Conclusion: It is frequent event that complication after gastrointestinal operation were accompanied by endotoxemia. Particular care of endotoxemia is needed in patients with colorectal cancer or patients with liver cirrhosis. Endotoxemia was frequently accompanied by peritonitis with high mortality. The prognosis was poor where endotoxemia tended to relapse.

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Endotoxin-Determination in Peritonitis and Intestinal Obstruction

E.P.M. Lorenz, F. Dobrowolski, H.v. Zünke, M.Häring
Surgical Department Klinikum Steglitz Free University of Berlin

In the framework of the presented investigation the practcableness

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of the chromogenic LAL-Test in blood of patients with important surgical illness like ileus and peritonitis was examined after heat/dilution treatment of the blood.
The LAL-Test was carried out perioperative (t=72 h) at 34 patients in age of 19 to 90 (six peritonitis cases, 14 ileus cases, 14 patients as a control).
it was shown, that the plasma possesses an amidolytic activity in spite of the heat/dilution method. The reliability of the LAL-Testis reduced also because of the observation that different plasma shows a variation of its amidolytic activity. For that reason the chromogenic LAL-Test allows only semiquantitative determination after a heat/dilution method. Therefore the comparison of the different publications seems to be problematic.
In this study it was found significant different concentrations between the three groups. The highest values were found in the peritonitis group with the mean value of 50 pg/ml.
The ileus group shows a mean value of 16 pg/ml, and the control cases 5 pg/ml.
In spite of the existing amidolytic activity these dates show that the performed test could be able to have a diagnostic relevance.

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PLASMA ENDOTOXIN LEVEL AND ORGAN FUNCTION IN SEPSIS. RESULTS OF A CLINICAL TRIAL. D. Nitsche*, C. Szeiki*
Dept. Gen. Surgery, Univ.-Hospital, D-2300 Kiel/Germany

To clarify the role of endotoxins in septic organ failure, a prospective trial was carried out to establish whether there is a correlation between the amount of plasma endotoxin and both organ function and the clinical course of abdominal sepsis.
Method: In 57 patients operated on for diffuse peritonitis organ function was monitored and plasma endotoxin concentration was measured every 12 h till the patients' conditions improved or until the patients died.
Results: All peritonitis patients had preoperatively significantly elevated plasma endotoxin levels. Postoperatively a correlation was found between endotoxin concentrations and the clinical course of peritonitis. In patients who survived, the plasma endotoxin level decreased continually to the detection limit(0.02 EU/ml). All patients whose endotoxin concentration continuously increased and exceeded a certain critical value died of multiple organ failure. In cases of prolonged endotoxemia, impairment of the lung-, kidney- and liverfunction was found. In most of these patients there was a correlation between plasma endotoxin level and the development of pulmonary failure (r=0.788), kidney failure(r=0.824) and liver failure (r=0.834).
Conclusion: Endotoxin is one of the factors in abdominal sepsis - probably the main factor - that causes organ failure either directly or indirectly.

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TWO TYPES OF SEPTIC SHOCK CLASSIFIED ACCORDING TO THE COMBINATION OF ENDOTOXIN AND EXTREMELY HIGH LEVELS OF CYTOKINES. S. Endo,[1] K. Inada,[2] K. Taki,[1] S. Hoshi,[1] M. Yoshida[2]. [1]Critical Care and Emergency Center, [2]Department of Bacteriology, School of Medicine, Iwate Medical University, 19-1 Uchimaru, Morioka 020, Japan.

It has been known that endotoxin and cytokines are related to the occurrence of septic shock. Especially, the role of cytokines has been noted. Therefore, we measured endotoxin, tumor necrosis factor α (TNFα), interleukin 1 α, β (IL-1α, β), IL-2 and IL-6 in the plasma of septic shock patients chronologically collected within a few hours after assessing the occurrence of shock. After plasma was processed by a perchloric acid method, which we developed, endotoxin was measured by the endotoxin-specific chromogenic test, Endospecy (Seikagaku Kogyo, Tokyo, Japan). Cytokines were measured by ELISA kits. Levels of cytokines, with the combination of TNFα + IL-2 (8 cases), or IL-1β + IL-6 (10 cases), were found to be extremely high at the onset of septic shock, as compared to levels of sepsis without shock. Endotoxin level was positive in all cases in the former, but not in 5 cases (50 %) in the latter. A strong correlation (r=0.81) was noticed between the levels of TNFα and IL-2 in patients with septicemia and septic shock. Neither the high level of IL-1 β or IL-6 correlated with occurrence of shock. Thus, we classified the septic shock as two types; endotoxin + TNFα + IL-2 type and IL-1β + IL-6 type.

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INCREASED LEVELS OF PLASMA ENDOTOXIN DO NOT CORRELATE WITH THAT OF CYTOKINES IN SEPTICEMIA. K. Inada,[1] S. Endo,[2] K. Taki,[2] S. Hoshi,[2] M. Yoshida[1]. [1]Department of Bacteriology, [2]Critical Care and Emergency Center, School of Medicine, Iwate Medical University, 19-1 Uchimaru, Morioka 020, Japan.

Endotoxin induces production of tumor necrosis factor (TNF) α and other cytokines in plasma in animals and volunteers and these cytokines are thought to be a major mediator of endotoxin responses. In this paper, we studied the relationship between levels of endotoxin and cytokines and cytokines with each other in septicemia. Underlying conditions were burns (22 cases) and perforation of gastrointestinal tract (15 cases). Heparinized blood of each patient was collected every day after admission or within a few hours after assessing the shock. Endotoxin-specific chromogenic limulus test, Endospecy (Seikagaku Kogyo, Tokyo, Japan) was used for measurement of plasma endotoxin. The plasma was pretreated by the new PCA method which we developed. Combination of these methods permitted a specific and highly sensitive measurement. Cytokines were measured by ELISA kits. The levels of TNFα, interleukin 1 β (IL-1β), IL-2, IL-6 more or less elevated in septicemic patient, but scarcely correlated with the elevation of endotoxin levels. This result suggested that cytokine productions were related with other stimuli or with production in the local region (focus of infection). It was also shown that the cytokine level was not correlated with other cytokine levels, except for that of TNFα and IL-2, and IL-1β and IL-6 at the onset of septic shock.

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MOF AND ENDOTOXEMIA AFTER DIGESTIVE SURGERY

Makoto IWATA M.D., Yukihiro YAJIMA M.D., Hajime YOKOI M.D., Takashi NOGUCHI M.D., Yoshifumi KAWARADA, M.D.,FACS, & Ryuji MIZUMOTO M.D. FACS, FACG.

1st Dept. of Surgery, Mie University School of Med. Tsu, Mie, JAPAN

The incidence of Multiple Organ Failure(MOF) was high and prognosis was extremely poor, in hepatectomy for liver cancer associated with cirrhosis.

During last 14 years at our clinic, MOF was observed in 40 cases after digestive surgery. The post operative endotoxemia, reticuloendothelial function, presence of infection, process of MOF and serum endotoxin were evaluated in MOF cases. Only one(3.0%) out of 33 patients with liver failure was recovered from MOF, but 5(71.4%) out of 7 patients without liver failure were survived. In MOF cases, endotoxemia was found in approximately 90% and level of plasma fibronectin was less than 150 ug/ml, but sepsis was found only 4(20%) out of 20 patients with liver cirrhosis.

Although in MOF cases with sepsis, both level of Toxicolor and Endospecy test elevated, only the level of Toxicolor test elevated in MOF cases of liver failure without sepsis. Sepsis and DIC were preceded by elevation of LTB_4 in early phase.

In summary, these tests might be useful for differentiation between sepsis and liver failure with level of plasma fibronectin.

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THE ROLE OF ENDOTOXIN IN THE INTRAPERITONEAL AND IN THE SYSTEMIC INFLAMMATORY RESPONSE IN PERITONITIS. U. Schöffel, A. Scheiger, E. Jacobs*, G. Ruf, M. Lausen, B.U.v.Specht and E.H. Farthmann.
Departments of Surgery and *Bacteriology, University of Freiburg, FRG

Escape of bacteria and bacterial products from the intestine into the peritoneal cavity induces both a local and a systemic inflammatory response. It is not clear, however, whether a spillover of bacteria or endotoxins into the systemic circulation is a prerequisite for a septic development. In a series of 51 consecutive patients with secondary peritonitis we evaluated the correlation between intraabdominal and systemic endotoxin levels, activation of cellular and plasmatic systems (TNFa, Ea, PI, C3a, FPA), and severity of disease. The expected correlation was found between the endotoxin concentration in the peritoneal exudate (LPSe) and the intraperitoneal bacteriology (gram-neg. vs. sterile exudate: $p < 0.0001$; gram-neg. vs. gram-pos. exudate: $p < 0.001$; gram-pos. vs. sterile exudate: $p=0.48$). Tendencies were detected when comparing LPSe with intraperitoneal TNFa values, length of history, and systemic inflammatory response. During the later course, the plasma endotoxin concentration (LPSp) seemed to correlate with the severity of the disease (SSS) and with the final outcome. There was no correlation between: LPSp and LPSe levels ($r=0.014$), TNFp and TNFe ($r=0.063$), LPSp and TNFp ($r=0.042$), LPSp/e and different etiologies, LPSp and the length of history ($r=0.157$), LPSp and bacteriology ($r=0.066$), LPSp and the systemic inflammatory response (activation score, $r=0.085$), LPSe and the local inflammatory response ($r=0.085$), LPSp/e and the severity of the disease (APACHE II: $r=0.069/0.063$; SSS: $r=0.075/0.083$; MPI: $r=0.026/0.072$), and LPSp/e and the outcome ($r=0.147/0.104$). These results do not support an early major role of endotoxin in determining the severity and the further course of an intraabdominal infection.

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PERIOPERATIVE KINETICS OF ENDOTOXIN AND ENDOGENOUS MEDIATORS IN PATIENTS WITH INTRA-ABDOMINAL INFECTION. E. Zadrobilek, R. Függer, H. Andel, V. Evstatieva, F. Schulz, and F. Lackner.
Departments of Anesthesia/Intensive Care and Surgery I, University of Vienna, A-1090 Vienna, Austria

Since there are no data available for the perioperative kinetics of endotoxin and endogenous mediators in patients with intra-abdominal infection, we studied 6 women and 4 men (mean age 50 yr; 8 survivors) undergoing primary surgical intervention for intra-abdominal abscess (n=4), permigration peritonitis (n=3), and necrotizing pancreatitis (n=3). Endotoxin was quantified using a modified limulus amebocyte lysate test (ultrafiltration, phenol water extraction, and chromogenic substrate); levels 10 EU/ml were considered elevated. Sequential (minimum 7) measurements were made over a 6 to 10 hr perioperative period. The mean of all endotoxin values was 24.0 EU/ml; individual lowest and highest levels averaged 2.8 EU/ml (range 0.3 to 6.7) and 56.8 EU/ml (range 27.3 to 113.4; $p < .001$, Wilcoxon test), respectively. We observed transient episodes of endotoxemia rarely related to surgical manipulation. The biological responses, measured in terms of tumor necrosis factor (immunoradiometric assay; range 3 to 703 pg/ml, mean 220) and interleukin-6 (enzyme-linked immunosorbent assay; range 0 to 16800 pg/ml, mean 116) release varied extensively. These findings may explain the results of recently published studies which demonstrate a poor correlation between actual concentrations of endotoxin (and released cytokines) and the severity of illness and the prognosis of surgical septic patients, respectively. Furthermore, we suggest that experimental models with single-bolus or continuous infusion of endotoxin do not authentically simulate intra-abdominal infection.

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COMPARISON BETWEEN CIRCULATING AND MONOCYTE (MO) ASSOCIATED CYTOKINES (CK) DURING SEPSIS SYNDROME. J. CARLET, C. MUNOZ, B. MISSET, C. FITTING, J.P. BLERIOT, A. CABIE, J.M. CAVAILLON (Spon Pr J.L. VINCENT) Intensive Care Unit, Hôpital SAINT JOSEPH and Unité d'Immuno-allergie, Institut PASTEUR, Paris, France.

Circulating IL-1, TNFα and IL-6 were searched in plasma and in MO lysates of 18 patients (pts) with sepsis syndrome (BONE Crit Care Med 1989) as well as 6 pts with non septic shock, at admission, every day until day 3 and then every week until day 21 or discharge. Circulating IL-1ß, TNFα, and IL-6 were detected in most pts either septic or not. The most frequent CK was TNFα (90% of the pts). Detectable levels were found for a long period of time. Maximum levels of IL-1ß (213±66pg/ml) and TNFα (187±19pg/ml) were significantly higher (<0.05) than those found at admission. Non survival pts had higher plasmatic levels of TNFα, IL-6 and lower levels of IL-1ß than survival ones, although a statistical significance was reached only for IL-6. TNFα levels were significantly higher in pts with ARDS and shock than in pts with either ARDS or shock alone or no organ failure. TNFα (388±59pg/10^6 MO) and IL-1ß (152±53pg/10^6 MO) were frequently found associated with MO lysates (89% and 56%, respectively) but no correlation exists between plasma and cell associated CK levels. IL-1α was found in the MO of only 29% of the pts. On the last sample available (14±1.5 days) out of 15 pts only 2 (12%) had still detectable plasmatic TNFα levels whereas 12 (80%) had MO associated TNFα (282±59 pg/10^6 MO). Because of the half life of the CK and the number of high specific receptors, it is likely that detectable plasma CK may represent the excess of CK which have not been trapped by the environmental cells.

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SERUM TUMOR NECROSIS FACTOR ACTIVITY IN HORSES WITH COLIC DUE TO GASTROINTESTINAL DISEASE. D.D. Morris, J.N. Moore and N. Crowe*
College of Veterinary Medicine, The University of Georgia, Athens, GA, 30602

Gastrointestinal (GI) diseases which cause colic are associated with high morbidity and mortality in horses. Most of the pathologic sequelae are due to endotoxemia. Since tumor necrosis factor (TNF) is an important proximal mediator of endotoxemia in other species, a study was performed to determine the prevalence of increased serum TNF activity in horses with colic. The association between serum TNF activity and prognosis for survival was also evaluated. Venous blood samples were collected from 289 horses presented to the University of Georgia with colic during a 24 month period. Serum TNF activity was determined by an in vitro cytotoxicity bioassay using WEHI 164 clone 13 murine fibrosarcoma cells. Causes for colic were determined by clinical and laboratory data, exploratory celiotomy or necropsy. Fifty seven (20%) of the horses had serum TNF activity greater than normal horses and 23 of these (8%) had markedly elevated serum TNF activity (≥ 10 U/ml). Mortality rate and prevalence of markedly increased serum TNF activity were much greater in groups of horses with intestinal inflammatory disorders or strangulating obstruction than in horses with nonstrangulating obstruction ($p < 0.01$). Of the 78 horses that died, 14 (18%) had serum TNF activity ≥ 10 U/ml, while only 9 (4%) of the 211 horses that lived had markedly increased serum TNF activity. The mortality rate of the group of horses with markedly increased serum TNF activity (61%) was significantly greater than the mortality rate of horses with normal or only mildly increased serum TNF activity

Notes

(24%; p < 0.001). Results of this study suggested a positive correlation between colic and serum TNF activity in horses and that GI inflammation and strangulating obstruction were more likely to be associated with serum TNF activity. Horses with markedly increased serum TNF activity had a much poorer prognosis for survival.

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ENDOTOXEMIA ELICITS INCREASED SERUM INTERLEUKIN 6 ACTIVITY IN HORSES. D.D. Morris, J.N. Moore and N. Crowe*
College of Veterinary Medicine, The University of Georgia, Athens, Georgia, 30602.

Endotoxemia associated with gastrointestinal disorders that cause colic and bacterial septicemia is the leading cause of morbidity and death in horses. Host-derived cytokines released during endotoxemia are important mediators of ensuing detrimental hemodynamic and metabolic changes. Increased serum concentrations of Interleukin 6 (IL-6) occur in a number of inflammatory states and have been implicated in the pathophysiology of endotoxemia in many species. The present study was performed to determine: 1) whether serum IL-6 activity was elevated in horses during experimental endotoxemia and; 2) any correlation between serum IL-6 activity and clinical or laboratory data. Six clinically normal adult horses were given endotoxin (30 ng/kg) in 1 liter 0.9% sterile NaCl solution IV during 1 hour. Six horses served as controls and received only 0.9% NaCl solution. The temperature, heart rate, attitude, white blood cell count, and serum IL-6 activity were determined at various times after endotoxin or NaCl administration. Concentrations of serum IL-6 activity were quantitated in a bioassay using the murine hybridoma cell line B13.29 clone B9 which is dependent upon IL-6 for survival. All measured data changed from baseline after the administration of endotoxin ($p<0.05$). There were no changes in clinical or laboratory data of the cortrol horses. Serum IL-6 activity was significantly increased from 1 through 8 hours after the start of endotoxin infusion, with peak concentration at hours 3 and 4. Changes in serum IL-6 activity were significantly associated with increased temperature after endotoxin infusion ($p<0.05$). Results of this study document that serum IL-6 activity is increased during experimental endotoxemia in horses and increased serum IL-6 was associated with fever. IL-6 may be involved in the pathophysiology of equine endotoxemia.

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PLASMA C-REACTIVE PROTEIN USED AS A PROGNOSTIC MARKER AND FOR MONITORING OF TRAUMATIZED PATIENTS.
Åke Lasson, Depts. of Surgery and Surgical Pathophysiology, Malmö General Hospital, Sweden.

The ultimate outcome in various severe trauma is mostly dependent on different complicatons occuring after the trauma. The aim of this study was to see if plasma C-reactive protein (CRP) could be used as a prognostic marker and to predict complications earlier than the clinical signs.
Material and methods: Plasma CRP-levels were followed for 7-10 days in patients with acute pancreatitis (n=55), after various elective surgical procedures (n=83) and after urgent surgery for peritonitis (n=16). Furthermore, admission samples were analysed in patients presenting with acute abdominal pain (n=208) due to various diseases. Levels, as well as specific biochemical changes in CRP were analysed.
Results: The admission plasma CRP level correlated well with the severity of the disease, especially in inflammatory disease and in malignant disease. The peak CRP level reached correlated well with the extent of the trauma. Daily monitoring of patients using plasma CRP levels could predict complications mostly 1-3 days earlier than the clinical signs/suspicion of a developing complication, irrespective of trauma studied or type of complication occuring (sepsis, pneumonia, cholangitis, local infectious complications). The CRP patterns indicating a developing complication were either a remaining CRP elevation or a second increase in CRP levels. In malignant disease, raised preoperative CRP levels were found in inoperable cancers and remaining high postoperative CRP levels were found in cancers not radically operated or developing early recurrent cancer.
Conclusion: The admission plasma CRP level correlated well with the severity of trauma, and can thus be used as a prognostic marker for various purposes. Daily monitoring of severily ill patients using plasma CRP measurements depict relevant complications 1-3 days earlier than clinical signs. High preoperative CRP levels predict inoperable cancer and remaining high postoperative CRP levels were found in patients developing early cancer recurrency.

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DRUG-MONITORING OF HIGH-DOSE METHYLPREDNISOLONE - A FEASIBILITY STUDY IN SEPTIC PATIENTS
A. Dietrich[1]*, E. Neugebauer[1], B. Bouillon[2]*, A. Lechleuthner[2]*, S. Saad[2]*; Biochem. and Exp. Divis.[1] and Surgical Clinic[2], II. Dept. of Surgery, Univ. of Cologne, Ostmerheimerstr. 200, 5000 Köln 91, FRG

Fixed dose regimens of high-dose methylprednisolone (MP) as used in two recent multicenter trials on sepsis and septic shock (NEJM 317: 653-665;1987) made us prove that there were tremendous individual pharmacokinetic variances which partly explain the failure of both studies (Circ Shock 31:235;1990). Only a few patients were within a defined target concentration range of 10-60 ug/ml. It was the aim of this study to test the feasibility of therapeutic drug-monitoring concepts in 10 patients with clinical signs of sepsis (inclusion criteria of VA Coop. Study Group). The dose regimen used was 4x30 mg/kg b.w.

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every 6 h: an upper limit of 60 ug/ml was not to be exceeded. 3 blood samples were taken in each interval at defined times and MP levels were determined by a fast HPLC-technique. Each determination took 30 min including time for calculation. Pharmacokinetic data were calculated for each interval with a plasma level prediction for the following dosage. Application of the subsequent dosage was only allowed when predicted MP levels did not exceed 60 ug/ml. The predicted levels from only 3 measurements/interval were later on compared with total kinetic analysis (50 blood samples). As a result, there was a good agreement (r=0.92-0.99) between predicted and measured levels for all intervals. Therefore our model is considered suitable for therapeutic drug-monitoring studies of steroids in patients with septic shock.

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TISSUE OXYGEN PARTIAL PRESSURE WITHIN SKELETAL MUSCLE IS HIGH IN SEPTIC PATIENTS WITH MULTIPLE ORGAN FAILURE

St. Weidenhöfer*, P. Boekstegers*, G. Pilz*, K. Werdan
Dept. of Internal Medicine I, Klinikum Großhadern, University of Munich, FRG

In order to determine whether the whole body oxygen extraction defect of septic patients is related to a specific impairment of peripheral tissue oxygenation, the oxygen partial pressure (pO_2) distribution within skeletal muscle was measured in 40 intensive care patients. Comparison of patients - defined by hemodynamic and score data - with sepsis and multiple organ failure (n=20), fever without sepsis (n=10) and low output failure (n=10) showed that mean oxygen partial pressure within skeletal muscle (MpO_2) was abnormally high in septic patients (mean=48,8 mmHg). The level of MpO_2 was similar in gramepositiv (n=9), gramenegativ (n=4) and fungoid (n=4) sepsis.

patients	MpO_2 (mmHg)	CI ($ml/min/m^2$)	LVSWI ($g*m/m^2$)	SVR ($dyn*s*cm^{-5}$)	Oxdel ($ml/min/m^2$)	Oxextr (%)
Sepsis (n=20)	48.8(8.5)	5.1(1.5)	38.5(22)	575(124)	662(140)	25(13)
Sepsis c.d. (n=9)	48.4(9.7)	4.0(0.5)	27.2(10)	634(66)	579(62)	28(12)
Sepsis w.o. c.d. (n=11)	49.0(7.8)	6.0(1.5)	49.9(22)	522(143)	737(244)	22(13)
Fever (n=10)	27.7(6.0)	2.9(0.5)	38.8(8.4)	1203(222)	438(83)	34(8)
Low output (n=10)	22.6(6.9)	2.2(0.2)	18.1(4.4)	1301(363)	312(27)	35(11)

Values are means +/-SD. c.d.=preexisting cardiac disease, w.o.c.d.=without c.d.

Conclusions: The main finding of the study was that mean skeletal muscle pO_2 (MpO_2) was significantly increased in septic patients in contrast to fever without sepsis and low output failure. Lower whole body oxygen delivery in septic patients with preexisting cardiac disease did not change the level of MpO_2. Our data suggest that an oxygen consumption defect within tissue rather than an impaired oxygen transport to tissue may account for the lowered oxygen extraction in sepsis.

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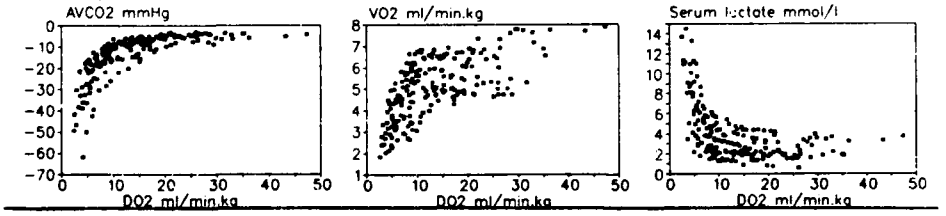
ARTERIOVENOUS PCO2 DIFFERENCES REFLECTS TISSUE HYPOXIA DURING HEMORRHAGIC SHOCK IN DOGS.

P Van der Linden*, J Bakker*, D. Schmartz*, JL Vincent.
Dept of Intensive Care, Erasme Hospital, Rt de Lennik 808, 1070-Brussels.

The present study tests the hypothesis that arteriovenous CO2 (AVCO2), like serum lactate reflects tissue hypoxia in controlled hemorrhagic shock in dogs. 12 dogs were anesthetized with isoflurane (1 MAC = 1.4 %), and mechanically ventilated. $\dot{V}O2$ was directly measured. O2 transport ($\dot{D}O2$) measured from thermodilution cardiac output, hemoglobin and SaO2 determinations. 30 min after splenectomy, $\dot{D}O2$ was reduced by progressive hemorrhage At each stage, $\dot{D}O2$, $\dot{V}O2$, serum lactate, and AVCO2 were measured. Critical $\dot{D}O2$ and $\dot{V}O2$ were 9.8 ± 1.1 and 5.6 ± 1.1 ml/min.kg, respectively. When critical DO2 was reached, serum lactate levels and AV CO2 started to rise exponentially (Fig 1-3) There was a significant linear relation between serum lactate and AVCO2 (r=0.82, p<0.001). Thus AVCO2 reflects tissue hypoxia as accurately as serum lactate levels in this dog model of hemorrhagic shock.



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EVIDENCE OF EARLY GUT HYPOXIA IN PIGS IN A NORMOVOLEMIC MODEL OF FECAL PERITONITIS.

I.Rasmussen* D.Arvidsson* and U. Haglund. Department of Surgery, Uppsala University, S-751 85 Uppsala, Sweden.

Oxygen consumption (VO2) may often be flow limited in septic patients despite supranormal cardiac output. The resulting ischemia can, especially in the splanchnic organs, induce changes that could be of critical importance for outcome. In this study we have followed oxygen delivery (DO2) and consumption (VO2) in the gastrointestinal tract by measuring oxygen content with intravascular light transmitting catheters (Oxymetrix Inc.). Furthermore, intramucosal pH (pHi) in the small intestine was cal-

Notes

culated using a tonometric catheter (Tonometrics Inc.) placed in the ileum. Animals were either subjected to fecal peritonitis (P, n = 5) at time 0 h or a sham procedure (C, n = 5). All animals were given crystalline fluid 40 ml/h x kg during preparation and 24 ml/h x kg during sepsis to ensure normovolemia as verified by repeated Hct measurements. Cardiac index (CI) and portal venous flow were measured with thermodilution. Arterial blood pressure remained constant in both groups.

		Pre Sepsis	1 h	2 h	5 h
CI	C:	129 ± 6	129 ± 4	130 ± 3	111 ± 4
	P:	130 ± 6	114 ± 6	88 ± 9*	83 ± 4*
G-i DO2	C:	6.7 ± 0.6	6.4 ± 0.7	6.0 ± 0.7	5.4 ± 0.1
	P:	7.6 ± 0.7	7.9 ± 0.9	5.6 ± 0.5	3.5 ± 0.3*
G-i VO2	C:	1.2	1.3 ± 0.3	1.2 ± 0.2	1.4 ±0.1
	P:	1.4	2.3 ± 0.3*	1.9 ± 0.1	1.7 ± 0.2
pHi	C:	7.32 ± 0.02	7.25 ± 0.05	7.29 ± 0.05	7.41 ± 0.02
	P:	7.27 ± 0.06	7.21 ± 0.08	7.10 ± 0.04*	7.12 ± 0.08*

Data are expressed as mean ± SEM. * indicates $p < 0.05$ vs control.
It is concluded that peritonitis increased gastrointestinal VO2 even if DO2 was reduced. However, pHi was rapidly reduced in peritonitis; before DO2 was reduced. The marked reduction in pHi indicates impaired gut oxygenation despite elevated VO2.

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GUT WALL pH DURING NORMODYNAMIC SEPSIS IN BABOONS. J. Davies, A. Schießer*, H. Redl* and G. Schlag*. Roodeplaat Research Lab., Pretoria, South Africa; * Ludwig Boltzmann Institute Exp. Clin. Traumatol., Vienna, Austria.

In endotoxemia and sepsis the gut is suspected to be an endogenous source of toxic material. A possible reason is malperfusion of the gut wall due to inadequate fluid supply, which could lead to gut wall damage. Therefore we have measured perfusion with a SMA flow probe, and gut as well as stomach wall pH by the indirect tonometer-technique. The experiments (8 hours) were performed in 6 male adult baboons subjected to E. coli bacteremia (5 x 10^8 CFU/kg infused over 2 hours).

Results: The E. coli infusion led to a normodynamic septic response (cardiac output at 2 hours + med 124 % (115 - 133), at 4 hours 99 % (86 - 135)) with a concomitant response of SMA flow (at 2 hours + med 118 % (72-164), at 4 hours 127 % (83 - 179)). This adequate perfusion of the splanchnic area resulted in a slightly decreased pHi (0 hours - 7.52 (7.47 - 7.54), 8 hours 7.37 (7.27 - 7.44), n = 5) of the gut wall with a similar trend of stomach wall pHi (0 hours - 7.38 (7.38 - 7.46), 8 hours 7.31 (7.30 - 7.37), n = 3).

Conclusion: Under our experimental conditions with a normodynamic septic response no (global) malperfusion in the gut area was seen; the gut wall pH was only minimally affected, which is in contrast to previously published results in a hypodynamic septic setting.

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INFLUENCE OF HEPATIC ISCHEMIA ON POSTOPERATIVE ORGAN FAILURE. Y. Shimahara, Y. Takada, H. Higashiyama, S. Iwata, K. Ozawa, S. Nomoto* and T. Ban* (spon: H. Hirawasa).
The Second Department of Surgery and *Cardiovascular Surgery, Kyoto University Faculty of Medicine, Kyoto, Japan 606.

Influence of hepatic ischemia in surgical fields was analyzed in relation to arterial ketone body ratio (AKBR) which reflects hepatic mitochondrial redox potential ($NAD^+/NADH$).

(1) Hemorrhagic shock after hepatectomy: The AKBR was decreased to below 0.7 at hemorrhage. In the patients with prompt recovery of the AKBR (within 3 days) to over 0.7 (n=9), liver failure was transiently observed in only 2 cases. The patients with delayed recovery (longer than 3 days) (n=5) suffered from liver failure (n=2), renal failure (n=3), respiratory insufficiency (n=2) and encephalopathy (n=1). The patients without recovery (n=4) died of MOF ultimately.

(2) Intraoperative hepatic ischemia in hepatectomy: Intraoperative AKBR dropped frequently to below 0.4 due to maneuvers such as hepatic vascular clamp, rotation of hepatic lobes, and hepatic hilar dissection which induce hepatic ischemia. In patients with decreased intraoperative AKBR (<0.4) for longer than 4 hours (n=9), marked postoperative abnormalities were observed in peripheral lymphocyte count, serum retinol binding protein, urine 3-methylhistidine and catabolic index, followed by significantly higher incidence of morbidity and mortality than other cases (n=52).

(3) Intraoperative hepatic ischemia in CPB (cardiopulmonary bypath) operation: CPB induces a shock with blood pressure of about 60mmHg, and the AKBR was decreased to below 0.4 (n=28). The cases with incomplete recovery of the AKBR (<0.4) (n=5) required intraaortic balloon pumping at ICU, while others with better recovery of AKBR (>0.4) (n=24) did not required it.

Conclusion: Ischemic liver induces a reduction of hepatic mitochondrial redox potential, and its recovery was closely related to occurrence of postoperative organ failure.

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THERE ARE NO HEMODYNAMIC DIFFERENCES IN ARDS PATIENTS WITH OR WITHOUT SEPSIS. J. Villar, J. Villalobos, J. Quintana, J.L. Manzano (Spon: Heinz Redl). Intensive Care Unit, Hospital del Pino, 35005 Las Palmas, Canary Islands, Spain.

Sepsis is the most common cause and complication of adult respiratory distress syndrome

Notes

(ARDS). To investigate whether sepsis contributes to the hemodynamic abnormalities, 32 patients with septic (n = 18) and non-septic (n = 14) ARDS of diverse causes were studied prospectively within the first 24 h of diagnosis of ARDS. We performed 78 serial hemodynamic determinations. There were no significant differences in age, level of PEEP, Apache II and therapy between the two groups. Mortality was higher in patients with septic ARDS (78% vs 57%), although this difference was not significant. In general, both groups had similar level of pulmonary hypertension (28±6 vs 25±4 mmHg) and elevated pulmonary vascular resistances (222±126 vs 188±61 d/s/cm^5). Although cardiac index (CI) and myocardial performance were increased over normal values, there were no differences in hemodynamics, ventricular function and oxygen transport between septic and non-septic patients nor between survivors and those who died. Non-septic patients showed a higher CI (4.1±1.4 vs 3.7±0.7 l/min/m^2), left ventricular stroke work (60±18 vs 55±20 grmts) and oxygen transport (936±231 vs 886±276 cc/min) than septic patients. Our data suggest that patients with septic ARDS have the same abnormalities in hemodynamics observed in patients with non-septic ARDS. These similarities might be explained by mechanisms common to ARDS rather than sepsis.

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PREOPERATIVE VALUES OF HEMATOCRIT AND BLOOD VISCOSITY EXPECT MULTIPLE ORGAN FAILURE FOLLOWING VALVE REPLACEMENT.

Tadashi Kato and Nobuko Tsushima.
Toyokawa City Hospital, Toyokawa, Aichi 442, Japan and National Cardiovascular Center Hospital, Suita, Osaka 565, Japan

When cardiac function is impaired after cardiac surgery, oxygen demand and supply in tissues becomes unbalanced. The ratio of hematocrit to blood viscosity may be used to track oxygen delivery. Increased hematocrit and/or decreased blood viscosity theoretically increases oxygen delivery, so that hematocrit and blood viscosity play an important role in supplying oxygen to tissues. These relations are intuitively obvious following valve replacement, because intravascular hemolysis is common in patients with prosthetic heart valves

Thirty-eight patients ranging in age from 20 to 70 years underwent cardiac surgery for valve replacement. One week after surgery, 12 patients were diagnosed as multiple organ failure (MOF), while 26 patients were convalescencing well. Blood viscosity measured at a shear rate of 94.5 sec^{-1} using a Couette type viscometer (Low Shear 30, Contraves) at 37^{0}C. Values of hematocrit and blood viscosity were significantly higher in patients without MOF than in patients with MOF. Blood viscosity had a good correlation with hematocrit values. Patients with low hematocrit and low blood viscosity developed into MOF. Oxygen delivery from a hemorheological point of view showed a ratio of hematocrit to blood viscosity indicated no difference between the two groups.

The results of the present study suggest that low hematocrit and low blood viscosity should be improved prior to surgery since patients with these low values tend to develop into MOF postoperatively, although there are many factors in addition to the low hematocrit and low blood viscosity which may have contributed to MOF.

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NEOPTERIN AND TNF-α: COMPARISON OF TWO MARKERS OF MONOCYTE/MACROPHAGE ACTIVATION IN A SEPTIC BABOON MODEL. W. Strohmaier, H. Redl, G. Leichtfried, E. Paul, S. Bahrami, J. Davies* and G. Schlag. Ludwig Boltzmann Inst. Exp. Clin. Traumatol., Vienna, Austria; * Roodeplaat Res. Lab., Pretoria, S.A.

Neopterin (NEO) and TNF-α are release products of stimulated mononuclear cells. In contrast to TNF-α, no physiological role for NEO has yet been described. We were interested to follow the time course of TNF and NEO release in the acute (A; 8 hours) and chronic (C; 72 hours) septic baboon model, which are based on a 2-hour E. coli infusion (see Schlag et al.). Additionally, we measured endotoxin (LPS) levels by a kinetic LAL technique.

Results and Conclusion

		LPS (ng/ml)	TNF (ng/ml)	NEO/CREA (nmol/μmol)
Acute (A)	n = 5	18429 (14.8)	8008 (0.1)	0.081 (0.04)
Chronic (C)	n = 8	12631 (20.5)	10167 (0.1)	0.307 (0.06)
Time of peak level (hrs)	A	1 - 3	2	8
	C	1 - 3	2	~24

Values are presented as medians, baseline values in parenthesis. LPS levels reflect the fast destruction and clearance of bacteria and consecutively LPS from the circulation. In both experimental set-ups TNF-α shows a pronounced peak at 2 hours and a return to baseline after 4 hours. NEO peak levels appear after 8 hours (A) and ~ 24 hours (C); they are reduced to half the maximum values during the observation period. We conceive that activation of monocyte/macrophage seems to occur in at least two steps, offering an explanation for the diagnostic usefulness of NEO in routine measurements due to reduced fluctuation.

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CHANGES IN GRANULOCYTE ELASTASE IN CRITICALLY ILL PATIENTS

Toshiaki Ikeda, Rie Fujita, Syouei Itoh, Akibumi Ohomi, Kazumi Ikeda and Atsushi Isshiki.
Department of Anesthesiology
Hachiouji Medical Center, Tokyo Medical College

Abstract

We measured granulocyte-elastase in α1-proteinase inhibitor complex (GEL-α1PI) in 31 patients and 24 dead on arrival(DOA) patients in order to evaluate clinically the relatioship between GEL-α1PI and respiratory morbidity and the prognosis of the patients. All patients admitted into the ICU were intravenously administered 300000 units of Ulinastatine per day for three days. DOA patients were given 300000 units of Ulinastatine with several kinds of catecholamines for resuscitation intravenously. The resuscitation(restarting of cardiac beat) rate of DOA patients was 33%. The coefficient of correlation between GEL-α1PI and oxygenation index was -0.37, and the coefficient of correlation between GEL-α1PI and respiratory index was 0.43. These results suggest GEL-α1PI plays the role of oxygenation of the lungs. We were unable to determine a limiting value of GEL-α1PI which could be used for predicting the patient prognosis.

Almost all DOA patients showed extremely high values of GEL-α1PI, but these values varied quite widely. Ulinastatine was found to decline the values of GEL-α1PI. We could not conclude that Ulinastatine effected the resuscitation rate.

GEL-α1PI might be a useful parameter to evaluate the severity of a critically ill patient's condition.

S12: Endotoxin and Cytokines

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THE ROLE OF TNF AND RELATED CYTOKINES IN SEPTIC SHOCK.

A. Waage, +A. Halstensen, *P. Brandtzæg, S. Steinshamn, T. Espevik.
Institute of Cancer Research, Trondheim, *Department of Pediatrics, Ullevål Hospital, Oslo, and +Department of Internal Medicine B, Bergen.

Endotoxin triggers endogenous production of mediators of which emphasis has lately ben placed on cytokines like TNF, IL-1 and IL-6.
TNF is an important mediator which is released early in the cascade reaction, whereas IL-1 and IL-6 represent successive waves of cytokines. IL-1 is probably a weak mediator alone, but its impact may be to potentiate the lethal effect of TNF. IL-6 may contribute to the acute phase response and other manifestations often observed in septic shock, but has by itself or together with TNF no toxic effect. The cytokines are markedly compartmentalized to the systemic circulation in septic shock. The complex interactions of cytokines will be discussed. There are many etiologies and variations of septic shock, and an important question is whether the role of TNF and the cytokine cascade is common to all categories of septic shock. The distinct release and role of TNF appear to be characteristic for septic shock in an organism which is not influenced by previous immunological or other types of stress. The role of TNF in other categories of septic shock remains to be clarified. We have further studied the role and production of TNF and IL-6 in relation to granulocytes in mice depleted of granulocytes. The production of these cytokines are increased in granulocytopenic mice.

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DYSREGULATION OF IN VITRO CYTOKINE PRODUCTION BY MONOCYTES DURING SEPSIS.

J-M. Cavaillon, C. Munoz, B. Misset, C. Fitting, J-P. Blériot, J. Carlet. Unité d'Immuno-Allergie, Institut Pasteur, and Intensive Care Unit, Hôpital Saint Joseph, Paris, France

The production by monocytes of interleukin-1α (IL-1α), IL-1β, IL-6 and tumor necrosis factor alpha (TNFα) in intensive care unit (ICU) patients with sepsis syndrome or non-infectious shock has been investigated. Plasma cytokines, cell-associated cytokines within freshly isolated monocytes and spontaneous as well as lipopolysaccharide (LPS)-induced *in vitro* cytokine production were assessed at admission and at regular intervals during ICU stay. TNFα was the most frequently detected circulating cytokine (90 % of all patients; maximum levels = 187 ± 19 pg/ml), and IL-6 levels correlate with outcome. Despite the fact that IL-1α is the main cytokine found within monocytes upon *in vitro* activation of cells from healthy individuals, it was very rarely detected within freshly isolated monocytes from septic patients, and levels of cell-associated IL-1β were lower than those of TNFα (152 ± 53 pg/ml vs 388 ± 59 pg/ml, respectively; p = 0.005). Cell-associated IL-1β and TNFα were not correlated with corresponding levels in plasma. Interestingly, at the end of the longitudinal study (day 14 ± 1.5), only 2/15 survival patients had plasma TNFα whereas 12/15 had still monocyte-associated TNFα."Spontaneous" *in vitro* release of IL-1β and TNFα by cultured monocytes from septic patients was significantly higher than that from healthy controls, and TNFα levels correlated with the presence of TNFα found within freshly isolated monocytes. Upon LPS stimulation, we observed a profound decrease

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of *in vitro* IL-1α production by monocytes in all patients, and of IL-1β, IL-6 and TNFα in septic patients compared to healthy controls. This reduced LPS-induced production of cytokines was most pronounced in patients with Gram negative infections. Whether this observation reflects an equivalent of the endotoxin tolerance described in animal models is under investigation. Finally, monocytes from survival patients, but not from non survival ones recovered their capacity to produce normal amounts of cytokines upon LPS stimulation. In conclusion, our data indicate an *in vivo* activation of circulating monocytes during sepsis as well as in non infectious shock and suggest that complex regulatory mechanisms can downregulate the production of cytokines by monocytes during severe infections, especially those involving Gram negative bacteria.

INVOLVEMENT OF LIPOXYGENASES IN THE FORMATION OF TUMOR NECROSIS FACTOR IN MURINE MACROPHAGES. U.F. Schade, R. Engel, J. Holler and D. Jakobs. Forschungsinstitut Borstel, D-2061 Borstel, FRG

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Lipoxygenase inhibitors are known to provide protection in experimental models of shock. We have tested several lipoxygenase inhibitors in endotoxic shock in mice. It was found that specific inhibitors of 5-lipoxygenases (synthesis of leukotrienes) were not protective, whereas compounds that blocked formation of 13-hydroxyoctadecadienoic acid (13-hydroxylinoleic acid, 13-HODD) increased survival. When, however, TNF-α was used for the lethal challenge, the lipoxygenase inhibitors were without effect. Lipoxygenase inhibitors prevented the formation of LPS-induced TNF in vivo and in vitro (macrophage cultures) and interfered with the formation of mRNA for TNF-α in macrophages. These results suggested an involvement of a lipoxygenase product in the LPS-induced formation of TNF. With the aim to identify the responsible lipoxygenase product, macrophages were hydrolyzed and analyzed for possible lipoxygenase products. 13-HODD was isolated and found to be significantly increased in LPS-stimulated macrophages. Exogenous 13-HODD (nonstimulatory per se), together with substimulatory amounts of LPS induced mRNA for TNF-α and TNF-bioactivity in the supernatant. From these results it is concluded that 13-HODD is of functional importance in the LPS-induced formation of TNF-α by macrophages.

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THE ROLE OF PHOSPHOLIPASE A_2 AS A MEDIATOR OF INFLAMMATION IN SEPTIC SHOCK **W. Pruzanski and P. Vadas, Inflammation Research Group, University of Toronto, Ontario Canada M4Y 1J3**

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The septic shock syndrome (SSS) is a common cause of death. To elucidate the pathogenesis of the SSS, various mediators of cardiovascular collapse have been sought. Endotoxin was found to elicit synthesis and release of TNF and IL-1. Since the mediators were found to be signals for the synthesis and release of phospholipase A_2 (PLA_2), and since PLA_2 was found to be strongly proinflammatory when injected into the skin, joints, or lungs of experimental animals, we postulated that the intravascular release of PLA_2 initiates events leading to cardiovascular collapse in SSS. We found that PLA_2 rises 11-fold after i.v. endotoxin challenge in rabbits and that it reproduces cardiovascular collapse when given i.v. to healthy rabbits. In retrospective and prospective studies of SSS in humans, circulating PLA_2 increased markedly (up to 200-fold). The level of PLA_2 correlated with the severity of circulatory collapse, incidence of ARDS and with disease outcome. In 23 healthy volunteers who received i.v. endotoxin, marked increase in circulating PLA_2 (up to 75-fold) followed the increase of TNF. Haemodynamic changes corresponded to the PLA_2 levels rather than to the maximum TNF. In 26 cancer patients, who received i.v. hrTNF, circulating PLA_2 increased markedly and reached maximum in 18-24 hrs. The levels of PLA_2 correlated to the dosage of TNF. These above experimental and clinical studies imply that induction of TNF release by endotoxemia subsequently leads to marked hyperphospholipasemia A_2 which may be responsible for some clinical and haemodynamic manifestations of SSS, which in the past were attributed to endotoxin and TNF.

COMPLEMENT ACTIVATION: A CRITICAL "TRIGGER" FOR THE SEPTIC RESPONSE. **D. Fry, University of New Mexico, Albuquerque, New Mexico, 87131.**

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The septic response has been characterized by an increase in cardiac output, a reduction in peripheral vascular resistance, and narrowing of arteriovenous oxygen differences. In previous experiments, these features of the septic state have been identified in peritonitis in the rat. Because complement activation via the alternative pathway has been

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identified as an event in bacteremia man, we have studied complement in cecal ligation-puncture peritonitis in the rat.

Summary of large numbers of experiments indicate that systemic activation of the complement cascade does occur in rat peritonitis. When zymosan was administered systemically, the rats developed a septic response characterized by increased cardiac output, reduced peripheral vascular resistance, and evidence of microcirculatory arrest in the hepatic microcirculation. Cobra venom activated complement to a greater degree than did zymosan but did not reproduce the septic response. However, the addition of platelet activity factor to cobra venom reproduced the septic response of zymosan, peritonitis, and endotoxin. Furthermore, complement depletion confers a relative protection to rats subjected to endotoxin challenge. From these data, it is concluded that complement activation is an important mechanism in "triggering" the septic response. Modulation of activation of complement may be of significance in control of the septic response.

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TNF-DEPENDENT ELAM-1 EXPRESSION AND IL-8 RELEASE IN BABOON SEPTICEMIA. H. Redl, G. Schlag, H.P. Dinges, W.A. Buurman*, M. Ceska** and J. Davies***. Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria; * Dept. Surg., Univ. Limburg, Maastricht, The Netherlands; ** Sandoz Research Institute, Vienna, Austria; *** Roodeplaat Research Laboratories, Pretoria, South Africa.

Leukocyte-endothelial interaction is the result of leukocyte activation, endothelial activation or a combination of both. We have previously looked for endothelial and phagocyte activation in hyperdynamic baboon septicemia (live E. coli 5 x 10^8 CFU/kg) by examining ELAM-1, an endothelial-specific adhesion molecule, IL-8 plasma levels as well as granulocyte elastase.The aim of the present study was to identify the mediator responsible for ELAM-1 expression and IL-8 release in vivo. ELAM-1 expression is strongly evident in septicemia, concurrent with high circulating TNF and LPS plasma levels, but not in septic baboons after TNF-antibody therapy (15 mg CB6/kg). IL-8 release was attenuated in the TNF-AB group (n = 4 1.1 (0.2 - 2.8) versus n = 4 11.5 (7.4 - 30.9) ng/ml in septic control animals 4 hours after E. coli infusion) and elastase plasma levels were significantly ($p < 0.06$) reduced from 127 (45 - 234) to 20 (18 - 23) ng/ml at 10 hours. With therapy there is no circulating TNF but there are still high LPS levels. Furthermore ELAM-1 and IL-8 are hardly detectable in post-traumatic situations, where both TNF and LPS are low or not detectable at all. The present study demonstrates that ELAM-1 expression and IL-8 release in baboon septicemia is primarily TNF-dependent, either directly or via a TNF-derived mediator.

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A CRUCIAL ROLE FOR CD18 AND NOT FOR ELAM-1 AND ICAM-1 IN TNF-α INDUCED NEUTROPHIL TOXICITY.

Wim A. Buurman, Eckhardt J.U. von Asmuth, Cees J. v.d. Linden. Dept. of Surgery, Univ. of Limburg, B.M.C., Postbus 616, 6200 MD Maastricht, The Netherlands.

TNF-α can incite neutrophil mediated endothelial cell damage, and H_2O_2 release. Using specific mAbs, we found that the beta chain of the CD11/CD18 integrin has a major role in both detachment of human umbilical vein endothelial cells (HUVEC) and H_2O_2 release by TNF-α activated human neutrophils. In contrast, no influence on TNF-α induced H_2O_2 release or endothelial cell detachment were observed, using mAb directed against CD11a, CD11b or CD11c, whilst anti CD11a and anti CD11b mAb reduced adherence under parallel conditions. These results suggested a role, other than mediating adherence, for the CD11/CD18 complex in TNF-α induced neutrophil toxicity. Further we investigated whether ELAM-1 and ICAM-1 influenced the ability of neutrophils activated with TNF-α , to detach endothelial cells, or to release H_2O_2. $F(AB')_2$ fragments of anti ELAM-1 and anti-ICAM-1 antibodies did not affect the damaging effect of TNF activated neutrophils on TNF activated endothelium. However, anti CD18 mAb remained inhibitory for the TNF-α induced increase in neutrophil mediated detachment and neutrophil H_2O_2 release, indicating that a specific CD11/CD18 involving signal, instead of adherence, triggers toxicity of TNF-α activated neutrophils. This study provides evidence for an essential role for CD18 and the absence of a role for ELAM-1 and ICAM-1 on endothelial cells, in inciting neutrophil induced endothelial cell damage, or neutrophil H_2O_2 release.

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REGULATION OF INFLAMMATORY CYTOKINES BY PGE2, DEXAMETHASONE, AND PENTOXIFYLLINE.

R. Strieter, T. Standiford, W. Scales, M. Rolfe, and S. Kunkel. University of Michigan Medical School, Ann Arbor, MI 48109-0360.

The pathogenesis of septic shock, ARDS, and multiorgan injury is related to production of an array of humoral mediators that can either potentiate or suppress the generation of specific cytokines, TNF, IL-1, and IL-8. The regulatory mechanisms that control TNF, IL-1, or IL-8 at the cellular and/or molecular levels remain to be fully elucidated. Our laboratory has been

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actively involved in the analysis of the regulation of TNF, IL-1, and IL-8 by the arachidonic acid metabolite (PGE2), glucocorticoids (dexamethasone; DEX), or pentoxifylline (PTX). We have shown that endotoxin (LPS)-stimulated peripheral blood monocytes (PBM) can synthesize PGE2 coincident with the production of TNF, IL-1, or IL-8. When PBM are treated with exogenous PGE2 and LPS, extracellular levels of TNF, IL-1, and IL-8 are reduced, suggesting a potential mechanism for autocrine control. The level of regulation is transcriptional for both TNF and IL-8, and post-translational for IL-1. In contrast, exogenous PGE2 and LPS stimulation of human alveolar macrophages (HAM) fails to inhibit either TNF or IL-8 production. Glucocorticoids are potent immunomodulators, DEX in combination with LPS suppresses both transcriptional IL-8 and TNF from PBM, but only transcriptional IL-8 from HAM. In addition, LPS and PTX a methylxanthine, inhibits immunologically activated murine macrophage-derived TNF at the transcriptional level. Furthermore, other methylxanthines and dibutyryl cAMP were shown to inhibit TNF at a transcriptional level. These data support: 1) mononuclear phagocytes derived from different compartments or stages of differentiation exhibit disparate responsiveness to immunomodulators, and 2) PTX and PGE2 exert their suppressive effect via the generation of intracellular cAMP.

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INTERCELLULAR COMMUNICATION IN HEPATIC FUNCTIONAL ALTERATIONS IN ENDOTOXEMIA. J.A. Spitzer, Dept. of Physiology, LSU Medical Center, New Orleans, LA 70112. USA

Cell-to-cell signal traffic has been invoked as a likely mechanism for stimulated hepatic glucose output following a short pulse of E. coli. endotoxin (ET) (JBC 263:6953, 1988) and PGE_2 production by Kupffer (K) cells in response to a septic stimulus (J Leuk Biol 47:304, 1990). This study was directed towards understanding the regulatory roles of some hepatic nonparenchymal (NP) cell products, i.e. eicosanoids and tumor necrosis factor (TNF) on parenchymal (P) cell metabolism and hormonal responsiveness in endotoxemia. Prior to cell isolation, ET was infused i.v. for 3 h through an indwelling catheter from an outside pump, or 30 h via a surgically implanted osmotic pump into Sprague-Dawley rats, 300-350 g BW. The eicosanoid profiles of elutriated K and endothelial (E) cell fractions of ET- and saline-infused rats were determined at the two time points. ET infusion resulted in a shift from predominantly cyclooxygenase to lipoxygenase products and an inversion of the PGD_2/PGE_2 ratio. The effect of conditioned medium (CM) prepared from supernatants of K cells of ET treated rats on gluconeogenesis (GNG) by P cells was evaluated. Coincubation of CM with P cells of saline-infused controls resulted in a depression of the rate of GNG that was about one half of the reduction induced by *in vivo* ET infusion. The stimulatory effect of $PGF_{2\alpha}$ on GNG in the perfused liver (Agents and Actions 31:3, 1990) was shown to be blunted by 30 h of continuous ET infusion. Infusion of human recombinant TNF into rats mimicked the previously noted perturbations due to ET infusion in P cell inositol lipid metabolism under basal conditions and upon vasopressin stimulation. Thus, several examples are provided of intercellular communication via lipid and protein mediators contributing to hepatic metabolic adjustments in endotoxemia. (Supp. by NIH grants GM 32654 and GM 30312.)

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HYPOXIA ENHANCES CYTOKINE PRODUCTION BY ALVEOLAR MACROPHAGES. J.M. Kisala*, A. Ayala, I.H. Chaudry. Department of Surgery, Michigan State University, East Lansing, MI 48824, USA.

The cytokines interleukin-1 (IL-1), tumor necrosis factor (TNF), and interleukin-6 (IL-6) are important mediators of the response to infection, inflammation, and injury. In the lung, the alveolar macrophage (Mø) plays an active role in immune function, including phagocytosis and cytokine production. The purpose of this study was to determine whether elaboration of cytokines by Mø is increased by hypoxia. Alveolar Mø were harvested from Sprague-Dawley rats by bronchial lavage. Peritoneal Mø were obtained by peritoneal lavage. Half of the cells from each animal were incubated in an anaerobic environment (95% N_2, 5% CO_2) for 1 hr; the other half were incubated aerobically. Endotoxin (1.0 μgm/ml) was then added and all cells were incubated aerobically for another 24 hrs. The supernatants were then removed and analyzed for IL-1, IL-6, and TNF by bioassay. The peritoneal Mø showed no difference in cytokine production by the aerobic and anaerobic cells. The anaerobically treated alveolar Mø demonstrated significantly increased production of IL-1 (31.4 ± 4.6 vs. 23.1 ± 4.1, $p < 0.001$) and TNF (1067 ± 262 vs. 695 ± 224, $p = 0.02$) when compared to the aerobically treated cells (all results expressed as units/10^6 cells ± SEM). There was no significant difference in IL-6 production (0.85 ± 0.21 vs. 0.70 ± 0.18, $p = 0.12$). These results indicate that hypoxia enhances cytokine production by alveolar Mø, possibly potentiating to the response to pulmonary pathology such as lung injury, atelectasis and pneumonia.

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SEPSIS-INDUCED ALTERATION OF MONOCYTE FUNCTION: ROLE OF FcR+SUBSET FOR THE REGULATION OF IL-6 SYNTHESIS IN HUMANS

M. Storck*, E. Faist *, R. Sendtner*, L. Hültner*[1], W. Ertel, F.W. Schildberg*

Department of Surgery and [1] GSF Institute, LM University of Munich, Klinikum Großhadern.

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Cytokines play an important role in the development of septic organ failure. Interleukin-6 (IL-6) is a pleiotropic cytokine which is produced mainly by macrophages (Mø) and is able to induce an acute phase reaction in hepatocytes. Recently, it has been possible to separate subsets of Mø with and without Fc Receptors (FcR+/FcR-). It was the aim of this study to analyze the profile of IL-6 synthesis in FcR+ and FcR- Mø fractions from septic patients to further characterize the altered functionality of Mø during septic challenge.
9 patients with postoperative sepsis (sepsis severity score: 10.7±0.9; mean ±S.E.M.) were compared with 6 healthy controls. Mø isolation was performed using elutriation technique. Cultures of $1x10^6$ cells were stimulated with LPS (C. parvum, 1000 ng/ml / 24h) and IL-6 release was determined using a specific bioassay (7 TD1 murine hybridoma cell line). The amount of Mø in parallel PBMC cultures was determined with LeuM3. FcR+ Mø were identified after rosetting with Anti-D labelled erythrocytes. Results: (x±S.E.M, * $p<0.05$ p vs.C)

	LeuM3 (%)	FcR+ (%)	Il-6/FcR+ (Units/ml)	Il-6/FcR- (Units/ml)
P	34.4±6.7*	34.4±4.1	3085±77*	2352±570*
C	10.5±2.3	21.6±3.2	634±131	1134±236

The percentage of FcR+Mø in septic patients was higher than in controls. The IL-6 production of FcR+Mø (3085±577) and of FcR-Mø (2352±570) from septic patients was significantly elevated above control values. The relation of IL-6 synthesis (FcR+ / FcR-) production was 1.31 during sepsis and 0.56 in healthy controls. We conclude, that FcR+ Mø are responsible for the altered Il-6 production in septic patients. Further analyses of altered monokine production of Mø subsets are necessary and will provide further understanding of the altered Mø function in sepsis.

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AUTOREGULATION OF MACROPHAGE TUMOR NECROSIS FACTOR PRODUCTION BY PLATELET ACTIVATING FACTOR. P. Bankey*, R. Singh*, and F. Cerra.
University of Minnesota, Dept. of Surgery, Minneapolis, Minnesota 55455

The macrophage cytokine Tumor Necrosis Factor (TNF) has been implicated as an integral signal of shock. Endotoxin signals the production and release of TNF; however, a number of inflammatory signals appear to alter TNF production including Platelet-activating factor (PAF). Since endotoxin has been demonstrated to signal both TNF and PAF production we hypothesize that PAF may be an autoregulatory inhibitor of TNF production. To test this hypothesis we determined the effect of PAF, the PAF antagonist BN 52021, indomethacin, and Prostaglandin E2 on endotoxin stimulated TNF production in vitro. Murine peritoneal macrophages were obtained by lavage and cultured overnight before measurement of TNF production after stimulation in parallel with LPS alone, LPS+ PAF inhibitor, LPS+ PAF, LPS+ Cyclooxygenase inhibitor, LPS+ PGE2. TNF-alpha was assayed using L929 cytolysis and standardized with murine rTNF-alpha.

Culture Treatment	Control	LPS-100ng/ml	LPS-10ug/ml
Medium Alone	0.2+/-0.2	4.6+/-1.1	11.4+/-2.3
BN 52021(10uM)	0.3+/-0.1	7.4+/-2.4*	15.6+/-4.4*
PAF (10ug/ml)	0.1+/-0.1	1.9+/-1.5*	6.7+/-2.8*
Indomethacin (1uM)	0.4+/-0.3	6.9+/-2.2*	13.6+/-3.9

*p<0.05 vs. Medium Alone by ANOVA and Tukey's test

Macrophages stimulated with PAF also demonstrated significant PGE2 production as measured by RIA which was inhibited by Indomethacin. These results demonstrate that PAF (10ug/ml) is inhibitory to LPS signaled TNF production and is supported by the BN 52021 inhibition of PAF increasing TNF production. Data that PAF signals PGE2 production and that the inhibition of PGE2 production increases TNF production suggests that PAF may inhibit TNF via the production of PGE2. Since PAF has been demonstrated to be synthesized in response to endotoxin we conclude that PAF is an autoregulatory inhibitor of macrophage TNF production which acts in part via signaled production of PGE2.

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THE PLACE OF TUMOR NECROSIS FACTOR AMONG THE MEDIATORS OF ENDOTOXIC SHOCK. T. Mőzes, F.J. Zijlstra*, J.P.C. Heiligers*, C.J.A.M. Tak*, S. Ben-Efraim*, P.R. Saxena*and T.L. Bonta*
Erasmus University Rotterdam, The Netherlands and Semmelweis Medical University Budapest Hungary (Spon: J. Hamar)

At present tumor necrosis factor (TNF), a macrophage derived cytokine, is considered the key mediator in the host's response to invasive stimuli either directly or indirectly by releasing secondary mediators, such as eicosanoids and/or platelet activating factor (PAF) The role of TNF, PAF and eicosanoids during endotoxic shock was investigated in anesthetized pigs receiving E. coli endotoxin (LPS) into the superior mesenteric artery over a 60 min period. TxB_2, 6-keto $PGF_{1\alpha}$ and LTB_4 concentrations in superior mesenteric vein, right ventricle and aorta were assessed by radioimmunoassay during 3 hr after the start of LPS infusion. Blood and serum concentrations of PAF and TNF were also determined (bioassays) in superior mesenteric vein and aorta, respectively. Eight of the 17 animals infused with LPS died within 30 min after commencement of LPS infusion (non-survivors)while the other 9 survived the experimental period of 3 hours, though in a shock state (survivors). In non-survivors no changes could be measured in either PAF or eicosanoid release. However, a marked increase could be detected in TNF release within 30 min after LPS infusion. A significant, though transient increase in concentrations of TNF, PAF and eicosanoids was also measured in survivors. The release of PAF, thromboxane and TNF reached the maximum level between 30-60 min of endotoxemia. Prostacyclin reached its peak between 60-120 min after the start of LPS infusion, while LTB_4 production between 120-180 min. Interestingly, the concentration TNF was less in survivors than in non-survivors. The role of TNF for rapid death seems to be dominant. On the other hand, its role in the shock observed in survivors is unclear, all the more because the other mediators were simultaneously elevated. Furthermore, the release of PAF proceeded the TNF production. These data suggest that endotoxic shock is not crucially dependent on one class of mediators.

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SENSITIZATION AND DESENSITIZATION TO THE LETHAL EFFECTS OF MURINE AND HUMAN rTNF IN THE MOUSE SYSTEM. P.G. Brouckaert,N. Takahashi, B. Everaerdt, C. Libert and W. Fiers
Lab of Molecular Biology, State University, Ledeganckstraat 35 B-9000 GENT (Belgium)

Tumor Necrosis Factor (TNF) is a cardinal mediator of the lethal effects of endotoxin and hence of septic shock. The outcome (death or survival) of a challenge with TNF will nevertheless depend highly on the presence or absence of other substances or conditions. So, Hu rTNF is not lethal in mice unless sensitizers are added. It is our purpose to improve the characterization and to study the mechanisms of such systems. We identified IL-1, LPS, GalN, RU486, Mu rTNF(which is lethal in mice) and some tumors as sensitizers and showed that the addition of an IL-1receptor antagonist could abolish the sensitization by IL-1 but not by the other factors. Sensitization was also reflected in the patterns of IL-6 induction. We also studied two models of desensitization. In the first, we tolerized mice against the lethal effects of TNF by multiple injections of low doses of TNF. We observed that the antitumor effectiveness was not affected in tolerized animals, that the tolerance was not due to an altered bioavailability of TNF and that its induction was inhibited by indomethacin. In the second, we prevent lethality by a pretreatment with IL-1 or TNF - but not IFN-g or LPS - 4 to 12 h before a challenge with Mu rTNF. This desensitization is not due to an altered bioavailability or a diminished binding to cells of TNF. It is dependent on macromolecular synthesis in the liver, but neither IL-6 nor glucocorticoids are involved. It is selective in that some effects such as IL-6 induction are not modulated. We conclude that manipulation of the sensitization/desensitization systems is a feasible way of modulating TNF-effects and may have the advantage of being more selective than a direct inhibition o the effects of TNF e.g. by TNF-receptor antagonists.

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RELATIONSHIP BETWEEN PLASMA TNFα AND MORTALITY FOLLOWING E COLI ENDOTOXOCOSIS IN THE RAT: PROTECTIVE EFFECT OF DEXAMETHASONE. E.F. Smith III, M.J. Slivjak*, K.M. Esser*. SmithKline Beecham Pharmaceuticals, plc, Depts. of Pharmacology and Cell Sciences, King of Prussia, PA.

The purpose of these studies was to investigate the relationship between E. coli endotoxin (LPS)-induced stimulation of TNFα formation and endotoxin-induced mortality in male Fischer 344 rats. Plasma TNF α concentrations were determined by an ELISA assay. Intravenous injection of 30 mg/kg E. coli LPS to anesthetized rats increased plasma TNFα in a time-dependent manner. Initial TNFα concentrations increased from <20 pg/ml prior to the injection of LPS, to 345±93, 8,440±878, 18,018±1,527, 17,197±1,449 and 6,558±686 pg/ml at 0.5, 1, 1.5, 2 and 2.5 hr, respectively (n=70): peak concentrations (i.e., C_{max}) were 24,303±1,639 pg/ml. LPS produced a dose-dependent increase in plasma TNFα concentrations from 0.001-0.01 mg/kg: doses of LPS greater than 0.01 mg/kg produced a maximal TNFα response. In endotoxemic animals, 7 day survival following injection of doses of 0.3, 1, 3, 10 or 30 mg/kg LPS was 100% (n=4), 100% (n=5), 100% (n=10), 30% (n=10), and 0% (n=10), respectively. Thus, the difference between the ED_{50} dose of LPS to increase plasma TNFα concentrations (i.e., approximately 0.03 mg/kg) and the LD_{50} dose (i.e., approximately 5 mg/kg) was >300 fold. Dexamethasone at doses from 0.01-10 mg/kg (i.v.), administered 24 and 1 hr prior to challenge with 30 mg/kg LPS, dose-dependently inhibited the LPS-induced increase in plasma TNFα concentrations: 10 mg/kg dexamethasone inhibited plasma TNFα by 95%, and improved survival from 0% to 100% ($p<0.01$). These results indicate that peak plasma concentrations of TNFα may not accurately predict mortality, although nearly complete suppression of plasma TNFα concentrations with dexamethasone provided complete protection against the lethal response of LPS in this model. These results suggest that other expressed mediators may also contribute to LPS-induced lethality.

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TUMOR NECROSIS FACTOR$_\alpha$ (TNF$_\alpha$) POTENTIATES OXIDANT-MEDIATED INCREASE OF ENDOTHELIAL PERMEABILITY. Y. Ishii, P.J. Del Vecchio, and A.B. Malik. The Albany Medical College, Albany, NY 12208 U.S.A. and Jichi Medical School, Tochigi 329-04 Japan.

We examined the effect of TNF$_\alpha$ "priming" of endothelial cells on the increase in endothelial permeability induced by H_2O_2, a neutrophil-derived oxidant. Bovine pulmonary microvascular endothelial cells (BPMVEC) were grown to confluence on a gelatin- and fibronectin-coated microporous filter. ^{125}I-albumin clearance across the monolayer was determined for 60 min. Though pretreatment with low dose of rhTNF$_\alpha$ (100 U/ml) for 6 h had no direct effect on albumin permeability, it enhanced susceptibility of BPMVEC to H_2O_2. H_2O_2 (10 μM), which alone also had no direct effect, when added to monolayers after 6 h pretreatment with TNF$_\alpha$ increased ^{125}I-albumin clearance rates (nl/min) more than three times (control:17±5, TNF$_\alpha$: 100 U/ml 6 h: 23±6, H_2O_2: 10 uM: 18±8, TNF$_\alpha$+H_2O_2: 61±12*; values are mean±SD, *$p<0.01$). We measured intracellular content of GSH and catalase in BPMVEC to determine the potential role of decrease in these antioxidants in increasing the susceptibility to H_2O_2. TNF$_\alpha$ treatment (100 U/ml; 6 h) decreased total GSH content (8.12±0.58 to 6.96±0.65 nmole/10^6 cells; $p<0.05$), but did not alter catalase activity. The decrease in GSH was associated with an increase in GSSG. We examined the role of GSH in the response by pretreating endothelial cells with 2 mM GSH for 6 h (which produced 60% of increase in GSH content). This prevented the potentiation of increase in permeability mediated by H_2O_2. We tested an effect of xanthine oxidase (XO) inhibitor, since XO is a source of oxidants which consume intracellular GSH. Pretreatment with 0.5 mM oxypurinol attenuated the synergistic effect of TNF$_\alpha$ and H_2O_2. We conclude that active oxygen species produced by TNF$_\alpha$ through XO activation consume intracellular GSH and the decreased "oxidant-buffering capacity" is responsible for the increased susceptibility of endothelial cells to oxidants.

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INTERLEUKIN 4 COUNTERACTS PYROGEN INDUCED ACTIVATION OF CULTURED HUMAN VASCULAR ENDOTHELIAL CELLS

S.Kapiotis, R.Eher, H.Strobl, J.Besemer, D.Bevec, P.Valent, P.Bettelheim, K.Lechner, and W.Speiser (Spon: H.Redl)
First Med.Dept. and Institute of Immunology, Univ.of Vienna, A-1090 Vienna, Austria and Sandoz Research Inst., A-1235 Vienna, Austria

Pyrogens such as IL-1, TNF and bacterial lipopolysaccharide(LPS) influence several endothelial cell(EC) activities within the blood coagulation and the immune system. These mediators cause a decrease in anticoagulant EC thrombomodulin(TM) surface activity and thus shift the EC surface into a procoagulant state. On the other hand IL-1, TNF and LPS play a role in the regulation of EC-leucocyte interaction. Under the influence of these pyrogens EC ICAM-1 surface expression is markedly enhanced. Both phenomena seem to be involved in inflammatory reactions at the vessel wall. In the present study the influence of IL-4, a product of activated T-cells, on pyrogen induced TM-downregulation and ICAM-1 upregulation was investigated. Coincubation of pyrogens and IL-4 in a dose dependent manner led to an antagonization of the pyrogen effects. After 15 hours of incubation IL-1(100U/ml), TNF(500U/ml) and LPS(10ug/ml) caused a reduction of TM activity to 43$\pm$11%, 46$\pm$15%, 36$\pm$12%($p<0.05$) of untreated cells. Coincubation of these mediators completely neutralized this pyrogen effect. After 72 hours of incubation, IL-1(100U/ml), TNF(500U/ml) and LPS(10ug/ml) caused an increase in ICAM-1 surface expression to 727$\pm$121%, 1274$\pm$93% and 1734$\pm$120% ($p<0.05$) of controls. Coincubation of pyrogens with IL-4(500U/ml) resulted in a significant reduction of ICAM-1 upregulation (210$\pm$45%, 710$\pm$85%, 500$\pm$89%). We conclude from our results that IL-4 expresses anticoagulatory and antiinflammatory activities on the vascular endothelium by limiting pyrogen induced downregulation of TM and increase in leucocyte adhesiveness.

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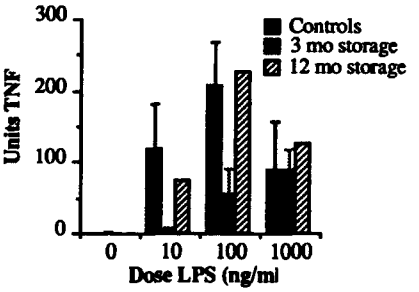
LIPOSOME ENCAPSULATED HEMOGLOBIN ALTERS ALVEOLAR MACROPHAGE RESPONSE TO LPS. L. Langdale, R. Maier, C. Rice. Veterans Affairs Med. Cnt. and Univ. of Wash., Seattle, WA 98108

Liposome encapsulated bovine hemoglobin (LEBH) is under development as a potential red cell substitute. While it has shown efficacy as an oxygen carrier, the impact of LEBH clearance by macrophages on cellular function has not been addressed. In addition, alterations due to storage and breakdown of the product have not been examined. Methods: Alveolar macrophages were lavaged from the lungs of Pasteurella-resistant New Zealand white rabbits. Harvested cells were suspended in RPMI, seeded at $5x10^6$ cells/well in 12-well flat bottom plates, and incubated at 37^{0}C for 3 hours in a 60:40 dilution (0.6 ml RPMI with 0.4 ml LEBH or RPMI). Cells were washed four times with RPMI to remove residual liposomes, then challenged with 10, 100, and 1000 ng doses of LPS (E.coli 055:B5, Sigma), and incubated overnight. L-929 cell-kill assays for TNF (Flick & Gifford) were performed on the supernatant of each well.

Results: Alveolar macrophages incubated with stable liposomes (LEBH stored less than three months) showed a marked decrease in TNF production for low, but clinically significant doses of LPS. Cells exposed to LEBH stored for twelve months showed an augmented TNF response when compared to fresh material, until high doses of LPS were employed.

Conclusions: TNF production by alveolar macrophages is altered after incubation with LEBH. Since the macrophages are capable of responding normally to high doses of LPS, the reduction in TNF observed at low doses suggests an inhibition of TNF production when exposed to intact liposomes. This protection is lost when the liposomes become unstable after prolonged storage.



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STIMULATION OF OXYGEN RADICAL RELEASE FROM HUMAN GRANULOCYTES BY CYTOKINES INVOLVED IN PATHOGENESIS OF SEPSIS

W. Macheiner, C. Oismüller, A.F. Hammerle and M. Micksche (Spon: G. Schlag)
Institute of Applied & Experimental Oncology, Univ. Vienna and Sepsis Research Group, Dep. of Anesthesia & General Intensive Care, Univ. Vienna, A-1090 Vienna

Recently great attention has been directed to the role of cytokines in a variety of clinical conditions including malignant and infectous diseases and also in septic syndrom. Increased levels of localy released and/or circulating cytokines such as tumor necrosis factor alpha (TNF-α), interleukins (IL-1, IL-6, IL-8), granulocyte-macrophage colony stimulating factor (GM-CSF), interferon gamma (IFN-gamma) are detected in patients with sepsis and might contribute to the activation of granulocytes. These cells respond to exogenous and endogenous stimuli (cytokines) by an increased production and release of oxygen radicals (respiratory burst). In the present investigation we studied the role of these cytokines in the regulation of granulocyte function. Human polymorphnuclear granulocytes (PMN) were isolated by dextran sedimentation and density gradient centrifugation. Respiratory burst activity (RBA) was determined in a chemiluminiscence assay. Stimulation of RBA was performed with fMLP, PMA and opsonised zymosan. In order to compare the effects of the different cytokines we measured RBA after different times of preincubation (0,1,2,4,6,9,12,15,18 hrs). All cytokines tested could not increase RBA spontaneously within the respective incubation periods. When RBA was stimulated with fMLP, PMA and opsonised zymosan a significant priming effect of cytokine pretreatment could be detected. Maximal priming effect on

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RBA was measured after 6 hrs of preincubation, whereas afterwards time kinetics revealed a decrease of this reactivity (bell-shaped curve). This study reveals that cytokines are able to augment RBA of PMN in a time dependent manner. Our data point to the importance of these cytokines in pathogenesis of septic syndrom.

SUPEROXIDE ANION RELEASE BY ALVEOLAR MACROPHAGES OF TUMOR NECROSIS FACTOR-(TNF) TREATED RATS. J.A. Spitzer and A.M.S. Mayer*, LA St. Univ. Med. Ctr., New Orleans, LA, 70112. **264**

Alveolar macrophages (AM) are the major resident inflammatory cells in the lungs. Besides performing important phagocytic and bactericidal functions for the removal of inhaled particles and bacteria, this cell type also has a significant regulatory role in the immune response of the lung. TNF, produced by macrophages, has a wide variety of physiological and immunological effects observed *in vitro*. TNF administration *in vivo* results in syndromes that mimic sepsis or endotoxic shock. TNF is thought to be a proximal mediator of the lung's inflammatory response against invading microbes. The purpose of this investigation was to assess the modulation by human recombinant TNF of superoxide anion (SO) release by AM. Male Sprague-Dawley rats (280-320 g. BW) were injected with 6 x 10^5U of TNF via the penile vein. Ninety minutes later, the animals were subjected to bronchoalveolar lavage to obtain AM.SO release by AM spontaneously and upon phorbol myristate acetate (PMA) and opsonized zymosan (OZ) stimulation was determined by superoxide dismutase inhibitable reduction of ferricytochrome c. PMA dose response studies revealed that upon PMA stimulation, AM of TNF-treated rats produced significantly more SO than did AM of saline control animals ($n = 4$ $p < 0.05$). Stimulation by OZ did not result in a consistent difference in SO production between AM of TNF- and saline-treated rats. AM lavaged 90 min. after intratracheal administration of 6 x 10^5U of TNF also showed hyperresponsiveness to PMA stimulation in terms of SO release. As demonstrated by Nelson, et al (J. Infect. Dis. 159: 189-194, 1989), TNF injected into the vascular compartment cannot be detected in the alveolar compartment. Nevertheless, we demonstrate the priming effect of i.v. injected cytokine in AM. Increased SO release can also be observed 90 min. after intratracheal administration of TNF. We hypothesize that the priming of AM observed *in vitro* of TNF-injected rats may be due to a soluble mediator(s) elicited by TNF and capable of cross-compartmental communication. (Supp. by ONR grant N00014-89-J-1916.) TNF was a gift from the Cetus Immune Corp.

COMPARATIVE EFFECTS OF *LIPOPOLYSACCHARIDE*(LPS) ON NEONATAL AND ADULT HEPATOCYTE/KUPPFER-CELL (KPFC) CO-CULTURES. DM Steinhorn*, FB Cerra. Univ. of Minnesota. Minneapolis, MN55455 **265**

As a model of cellular response to LPS, newborn (NB) and adult (AD) rat hepatocytes were harvested by collagenase digestion and cultured for 24 hours. Subsequently, NB and AD KPFC were harvested by enzymatic digestion and were added to the existing hepatocyte cultures in a ratio of 1:1. NB and Ad Kuppfer cells were also cultured alone and stimulated identically with LPS in dosages of 0.01, 0.1, 1.0 and 10 mcg/ml to assay for production of TNF and IL-1 by standard bioassay. AD and NB hepatocytes were co-cultured in triplicate with KPFC from both similar and opposite aged subjects for 24 hours at which time LPS was added as above. After 24 hours, the co-cultures were incubated with ^{3}H-Leu to assess total protein synthesis by the hepatocytes. The differences in protein synthesis between AD and NB co-cultures were submitted to an ANOVA with LPS concentration as a covariate. The decrease in protein synthesis was similar in magnitude between NB and AD co-cultures at all LPS dosages. There was a significant dose response to LPS for both NB and AD systems. Equal effects were seen with KPFC of opposite ages; although, adult KPFC produced significantly more TNF ($p=0.001$) and IL-1 ($p=0.021$) than NB KPFC. Both NB and AD KPFC demonstrated a dose response at differing LPS concentrations for TNF and IL-1 production. We conclude that significant differences exist in the cytokine response of neonates vs. adult rats in response to LPS. The effects upon hepatocyte protein synthesis are similar between the groups suggesting that mediators other than TNF and IL-1 are responsible for the hepatocyte responses seen in this system. These as yet unidentified mediators may account for the differences seen between mature and immature subjects in response to Gram-negative sepsis.

EFFECTS OF IL-1β PEPTIDE (ISP) FOLLOWING SURGICAL STRESS ON MINERAL METABOLISM: INFLUENCE OF ZINC STATUS. **M.A. Dubick*, R.H. Dressendorfer* and C.L. Keen*** (Spon: C.E. Wade)
Letterman Army Inst. Res., San Francisco, CA 94129, Highland Univ., Las Vegas, NM 87701 and Univ. of Calif., Davis, CA 95616, USA. **266**

IL-1β, an important mediator in the response to injury and infection, is known to affect Zn and Fe metabolism. Since Zn metabolism is altered by injury and trauma and Zn deficiency retards healing, we have begun investigations into cytokine-nutrient interactions following trauma or stress. The present study assayed tissue trace element concentrations in groups of rats fed diets either marginally deficient (5 ppm) or sufficient (50 ppm) in Zn. After 2 wks, a standard laparotomy with manipulation of the gut was performed as a model of surgical stress. At this time rats were infused via an osmotic minipump, with IL-1β immunostimulatory peptide (ISP) (20ng/h) or saline for 7 days. Marginal Zn deficiency was confirmed by

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plasma and liver Zn and liver metallothionein (MT) concentrations. In both ZD and C rats plasma Cp was stimulated by surgical stress and ISP administration, although activity was consistently higher in C than ZD rats. Surgical stress altered liver and kidney Cu, Zn, Mn and Fe concentrations, as well as, MT but specific trace element responses differed in C and ZD rats. In C rats, effects of ISP administration, alone, were not observed if the rats were stressed, whereas in ZD rats, trace element and MT concentrations appeared to reflect both the stress and ISP treatment. These data indicate that trace element metabolism following surgical stress and ISP treatment can be differentially affected by concomitant Zn deficiency. It remains to be established whether IL-1β or its specific peptides can overcome effects of marginal nutritional deficiencies in modulating healing.

S13: Update of Metabolism

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ADVANCES IN MUSCLE METABOLISM IN TRAUMA. Robert R. Wolfe, Farook Jahoor, David H. Herndon, Thomas Kimbrough, Dennis Gore.
The University of Texas Medical Branch and Shriners Burns Institute, Galveston, TX 77550

Muscle wasting has long been recognized as an important metabolic consequence of the response to severe injury, yet few studies have focused attention on human muscle metabolism in injury. We have therefore performed a series of studies in severely burned children and normal volunteers assessing the response to injury and the role of hormones (insulin, glucagon, growth hormone, catecholamines) in mediating the response. Both accelerated protein breakdown and impaired synthesis contribute to depletion of muscle protein after injury. The inhibition of synthesis can also be induced in normal volunteers by the infusion of catabolic hormones (glucagon, catecholamines), but the ability of insulin to suppress breakdown is retained. The depletion of some intracellular amino acid pools, particularly glutamine, may play an important role in the relative impairment in synthesis after trauma. Normalization of the catabolic hormone concentration in burn patients does not, however, completely normalize muscle metabolism.

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SIGNIFICANCE OF IMPAIRED HEPATO-MUSCULAR RELATIONSHIP IN SPETIC CATABOLISM IN DECREASED HEPATIC MITOCHONDRIAL REDOX POTENTIAL. K. Ozawa, Y. Shimahara and T. Kiuchi.
The Second Department of Surgery, Kyoto University Faculty of Medicine, Kyoto, Japan 606.

Peripheral protein degradation in muscles has an important physiological role as a substrate supply to central organs represented by the liver, and enhanced protein catabolism after trauma and sepsis is essential, if not excessive, to survival by promoting wound healing, immunological defense and vital organ function. The metabolic failure of liver is being recognized as an important factor in the progress of multiple organ failure. However, the role of muscle protein in this metabolic failure is not yet clarified. To estimate the contribution of muscle protein in whole body protein catabolism, muscular contribution index (MCI; urine 3-methylhistidine / urine total nitrogen) was determined in 49 cases of surgical patients, together with arterial blood ketone body ratio (AKBR) which reflects hepatic mitochondrial redox potential. MCI increased after operation and in severe infection, provided AKBR was maintained at above 0.7. MCI in septic patients, however, decreased dependently upon AKBR ($n=33$, $p<0.01$). To the contrary, plasma proteolysis inducing activity (PIA) determined by in-vitro bioassay increased in inverse proportion to AKBR ($n=20$, $p<0.01$). Plasma amino acid concentration in these patients markedly increased when AKBR decreased to below 0.4, not only aromatic but branched-chain amino acids ($n=23$, $p<0.05$). These results suggest that the substrate transfer from muscles to liver in septic cases fails to meet the demand under reduced hepatic mitochondrial redox potential, despite the rapid increase in the PIA. This finally leads to the failure of amino acid uptake by muscles as well as liver.These ruins in substrate exchange may form a metabolic background of multiple organ failure, which is often preceded by reduced AKBR.

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MUSCLE PROTEIN TURNOVER IN SEPSIS AND INJURY. M.J. Rennie
Department of Anatomy & Physiology, The University, Dundee DD1 4HN, Scotland

Skeletal muscle wasting accompanies septic shock but the mechanisms involved are unclear. With the use of stable isotope tracers to reveal the components of the net tissue amino acid fluxes, progress is beginning. In

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chronic wasting (eg, immobilization, cancer, malnutrition, etc) muscle protein synthesis is depressed with little evidence of elevation of breakdown; however in sepsis, major injury or burn, it may be elevated, with a variable response of protein synthesis (ie +, -, or =). Currently much interest focusses on the regulation of intramuscular glutamine, the high trans-membrane distribution ratio of which falls in sepsis and other conditions; the increase in efflux seems to be associated with changes in glutamine-Na+ transporter kinetics and in E_M and $[Na]_i/[Na]_o$. Glutamine has protein anabolic effects in muscle in man and its provision glutamine ameliorates net negative nitrogen balance in injured and septic patients. Strategies for metabolic intervention during the care of patients with septic shock now seem possible.

INTERCELLULAR MECHANISMS OF NET PROTEIN DEGRADATION IN SKELETAL MUSCLE DURING SEPSIS. **270**
Mohammed M. Sayeed, Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153, USA

Increased protein degradation in skeletal muscle is a commonly occurring metabolic response during sepsis. Although it provides amino acids for the hepatic acute phase protein synthesis, its persistence beyond a crucial level can lead not only to a depletion of the protein reservoir but also to muscle wasting. Recent studies have implicated roles of Interleukin-1, a proteolysis-inducing factor, prostaglandin E2, and/or catabolic hormones in the sepsis-related increase in skeletal muscle protein degradation. However, intracellular mechanisms for the increased protein breakdown during sepsis are unknown. We have investigated the involvement of sepsis-related alterations in cellular Ca^{2+} regulation and the intracellular proteases in the augmentation of protein degradation in skeletal muscle. We assessed also the role of an increase in Ca^{2+} influx into the skeletal muscle on the heightening of the protein degradation process. Sepsis was induced in male Sprague-Dawley rats by implanting into their abdomens bacteria laden (Escherichia coli and Bacteroides fragilis) pellets made out of sterilized feces. Sham rats were implanted with sterilized pellets without any bacteria. The febrile response and blood lactate concentrations were monitored to assess sepsis. Protein degradation, ^{45}Ca flux and proteolytic enzymes, calpain and cathepsins, were determined in isolated leg muscles. ^{45}Ca measurements assessed alterations in Ca^{2+} influx as well as Ca^{2+} content of the intracellular compartment(s). Compared to sterile-implanted rats, protein degradation and Ca^{2+} flux were significantly elevated in septic rats on days 1-3 post-implantation. Also, sepsis caused a significant elevation in the Ca^{2+}-dependent protein calpain but not lysosomal cathepsins. The exposing of control rat muscles to Ca^{2+} ionophore lead to augmentation of both Ca^{2+} influx and protein degradation. These studies have supported the concept that sepsis-related alterations in intracellular Ca^{2+} regulation and related Ca^{2+}-dependent proteases contribute to increased skeletal muscle protein degradation in sepsis.

GLUCOSE-TRANSPORTER (GT) mRNA ABUNDANCE IN MUSCLE, FAT AND LIVER DURING THE HYPOGLYCEMIC PHASE OF ENDOTOXIC SHOCK. **271**
W.P. Zeller, S.M. The*, M. Goto, M. Sweet*, M.E. Gottschalk*, R.M. Hurley, J.P. Filkins and C. Hofmann*, Depts. of Pediatr., Physiology, Surgery, Biochemistry, Loyola Univ. of Chicago, The Shock Trauma Inst., Stritch School of Med., Maywood, IL 60153 and Hines VA Research Services, Hines, IL 60141, USA.

Glucose transport is altered in endotoxic shock. GT proteins are responsible for cell specific uptake of glucose and therefore may be altered in endotoxic shock. We investigated the abundance of mRNA for GLUT 1, 2, and 4 in soleus muscle, epididymal fat and liver in endotoxic shock. Male Sprague-Dawley rats were treated with S. enteritidis lipopolysaccharide (LPS, 40mg/kg IP) or an equal volume of saline in controls. Tissues were rapidly harvested 4-6 hours after the injection. When the tissues were harvested, endotoxic rats and time matched controls revealed plasma glucose (111±4 vs 44+6mg/dl $p < 0.001$), insulin (40+9 vs 50+12 uU/ml), and lactic acid (1.3+0.1 vs 5.9+0.5 mM/L $p < 0.0001$) concentrations, respectively. In the table below, altered abundance of GT mRNA in tissues of LPS-treated rats is expressed as % saline-treated control.

Treatment	GLUT1			GLUT4		GLUT2
	Muscle	Fat	Liver	Muscle	Fat	Liver
Saline n=9	100%	100%	100%	100%	100%	100%
LPS n=12	314±29*	660±97*	871±207*	133±7*	133±31	58±13*

$\bar{x}$±SEM, *$p < 0.05$

At the time of hypoglycemia and lactacidemia, GLUT1 mRNA increased 3-fold in soleus muscle, 6-fold in fat and 8-fold in liver. GLUT2 in liver decreased, and GLUT4 in muscle increased significantly. Altered GT gene expression may underwrite the pathogenesis of hypoglycemia during endotoxic shock.

TRANSLATIONAL REGULATION OF PROTEIN SYNTHESIS IN SKELETAL MUSCLE OF SEPTIC RATS. **272**
T. C. Vary. Department of Cellular and Molecular Physiology, The Milton S. Hershey Medical Center, School of Medicine, Hershey, PA 17033

The rate of protein synthesis in vivo was measured by incorporation of

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phenylalanine into protein following anbolus administration of [^{3}H]-phenylalanine in skeletal muscle from control, sterile inflammatory and septic rats. An intraabdominal abscess developed following the introduction of a fecal-agar pellet inoculated with either saline (sterile inflammation) or E. coli and B. fragilis (sepsis) for five days. Animals were weight-matched to account for changes in body mass due to the septic process. The amount of protein present in skeletal muscle from septic animals was significantly reduced to 65% of the control value. Protein synthesis was not altered in sterile inflammation but was significantly reduced by 50% in sepsis. Total RNA content was not different in any of the conditions examined. The translational efficiency, expressed as protein synthesis/RNA, was not different in sterile inflammation but was significantly reduced by 50% in skeletal muscle of septic animals. Changes in the translational efficiency have been correlated with alterations in the level of ribosomal subunits. The amount of RNA in 40S and 60S ribosomal subunits were significantly increased in sepsis but not sterile inflammation. An accumulation of ribosomal subunits associated with a decreased efficiency of translation would indicate that a restraint in peptide-chain initiation had developed in skeletal muscle of septic animals. (Supported by NIH grants K04 GM00570 and R01 GM39277).

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EFFECT OF ANOXIA, FASTING, AND ENDOTOXIN ON 1,2-DIACYLGLYCEROL AND CERAMIDE LEVELS IN RAT LIVER AND SKELETAL MUSCLE IN VIVO. J. Turinsky, B.P. Bayly* and D.M. O'Sullivan*. Albany Medical College, Albany, NY 12208

The role of 1,2-diacylglycerol in transmembrane signaling resulting in stimulation of protein kinase C is well established. Since insulin increases 1,2-diacylglycerol levels in cultured myocytes, it has been proposed that 1,2-diacylglycerol could be a mediator of insulin-induced glucose uptake by muscle. However, we were unable to observe any changes in skeletal muscle 1,2-diacylglycerol levels in vivo during the period when insulin or exercise augmented glucose uptake by muscles of the rat several fold (J. Biol. Chem. 265:7933, 1990). Furthermore, we have shown elevated levels of 1,2-diacylglycerol in insulin-resistant tissues of the rat in vivo, suggesting that a long-term elevation of tissue 1,2-diacylglycerol concentration may contribute to the development of some types of insulin resistance (J. Biol. Chem. 265:16880, 1990). The aim of the present study was to search for other conditions associated with altered tissue levels of 1,2-diacylglycerol and thus identify those states in which 1,2-diacylglycerol could have a regulatory role in vivo. 1,2-Diacylglycerol was measured in liver and skeletal muscle of rats under three conditions: (1) during anoxia lasting up to 10 min; (2) during fasting for up to 6 days; and (3) during stress induced by injection of bacterial endotoxin. 1,2-Diacylglycerol was assayed with diacylglycerol kinase. In addition, ceramides were measured with the same assay. Anoxia induced a sustained 52-58% increase in liver 1,2-diacylglycerol and increases of 9-16% in liver ceramide levels. Muscle concentrations of 1,2-diacylglycerol and ceramides were not influenced by anoxia. Fasting for up to 6 days had no effect on 1,2-diacylglycerol and ceramide levels in liver, but increased skeletal muscle concentrations of 1,2-diacylglycerol and ceramides 88% and 44%, respectively. Injection of endotoxin resulted in transient 52-66% increases in liver concentrations of 1,2-diacylglycerol and ceramides, but had no effect on lipids in muscle. It is concluded that (1) anoxia increases 1,2-diacylglycerol and ceramide levels in liver; (2) 1,2-diacylglycerol and ceramides may play a role in metabolic adaptations in skeletal muscle during fasting; and (3) 1,2-diacylglycerol and ceramides are involved in the response of the liver, but not muscle, to endotoxin-induced stress in vivo. (Supported by NIGMS grant GM-22825)

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HEPATIC GLUCOSE PRODUCTION AND CALCIUM MOBILIZATION DURING HEMORRHAGIC SHOCK. Subir R.Maitra, Wansong Pan*, Evan R. Geller. Division of Trauma, Department of Surgery,SUNY @ Stony Brook, Stony Brook, New York 11794-8191.

Prolonged hemorrhagic shock (HS) is characterized by failure of compensatory mechanisms. Specifically, the initial hyperglycemia seen in shock is followed by hypoglycemia and resuscitation failure. The mechanism of this transition is unknown. The present study evaluated hepatic glucose production (HG) and intracellular free Ca^{2+} concentration (Ca^{2+})$_i$ during the hypoglycemic phase of HS. HS(n=8) was induced in fasted, anesthetized rats by reduction of blood pressure to 40mM Hg for 150 mnutes. Blood samples were then obtained for determination of plasma glucose (PG) and lactate (PL), from HS and control (C)(n=11) rats. Liver perfusion procedures were employed to measure the (HG) with and without added lactate. In a separate experiment, (Ca^{2+})$_i$ in hepatocytes was measured using the Ca^{2+} selective indicator Fura-2 from HS(n=5) and C(n=5) rats.

			HG(uM/gm/hr) Lactate Perfusion(mins)				[Ca^{2+}]$_i$(nm)
	PG mg/dl	PL mM	0	5	10	15	
C	149.6	1.0	11	16.6	20	20.9	175.8
	±13.4	±0.09	±0.8	±1.3	±1.2	±1.9	±45.6
HS	58.4*	6.6*	9.9	10.9	16.6	14.2*	481.6*
	±13.9	±0.8	±1.4	±2.7	±2.9	±3.7	±110 (*p<.05 compared to C)

HS was associated with hypoglycemia and hyperlacticacidemia. In HS rats, HG in response to lactate is attenuated and hepatocyte (Ca^{2+})$_i$ is elevated. We conclude that substrate specific stimulation of gluconeogenesis and (Ca^{2+})$_i$ is altered in the livers of rats subjected to HS.

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IMPORTANCE OF HEPATIC GLYCOGEN FOR SURVIVAL AFTER HEMORRHAGE 275

A Alibegovic and O Ljungqvist
Dept of Surgery, Karolinska Hospital, 104 01 Stockholm, Sweden

Already 24h food deprivation increases mortality after experimental hemorrhage. In the present study, the importance of available hepatic glycogen *per se* for survival in hemorrhage was investigated.

Methods. Adult male Sprague Dawley rats, food deprived for 24h, were anaestehstized and given a 3h infusion (0.3 ml/h/100g b.wt) of 0.9% NaCl (n =10) or 30% glucose (n = 10) before hemorrhage. The animals were subjected to 60 min hemorrhagic hypotension resulting in a 42% blood loss. After hemorrhage, animals were allowed free access to food and water, and observed for seven days. Separate animals (n=6, each group) were sacrificed prior to hemorrhage for hepatic glycogen analysis. Differences calculated using Mann Whitney U-test, or Fishers exact test.

Results.

(mean±SEM)	Liver glycogen (µmol/g)	Final glucose (mmol/l)	Final hematocrit (%)	Survival (%)
0.9%NaCl	104±32	5.2±0.1	40±1	0
30% glucose	578±72	16.1±0.9	35±1	100
p	<0.01	<0.01	<0.01	<0.01

Conclusions. Improved hepatic glycogen availabilty before hemorrhage can determine survival. Survival was closely related to improved plasma refill casued by hyperglycemia.

CHANGES IN THE CONTRACTILE FORCES OF THE RED/WHITE MUSCLES OF THE RAT FOLLOWING ISCHEMIA AND REPERFUSION. 276

K. KANTAS - J. HAMAR. - L. EGRI - J. RUPNIK
National Institute of Traumatology, Budapest, Ungarn

The purpose of this study was to investigate the functional status of different types of skeletal muscles (red or white) in the ischemia/reperfusion model of the anesthetized rat. Male Whistar rats were subjected to unilateral hindlimb ischemia (tourniquet) for one and two hours. Isometric force measurements were carried out on the ischemic and on the contralateral (control) sides at 0, 15, 30, 60, 90, 120 min, and one and two weeks after the start of the reperfusion (tourniquet release). Contractile forces of the extensor digitorum longus (EDL) and of the soleus muscles (SM) were measured in situ by a strain gauge connected to the distal tendon of the muscles. Muscle contractions were elicited by sciatic nerve stimulations. Following one and two hours of ischemia isometric tension varied between 0.2 and 36 % and 0 and 13 % of control, respectively, during the first 120 min of reperfusion. Contractile force of the EDL was 4 %, 0.6 % (1 day), 86 %, 60 % (1 week), and 80 %, 60 % (2 weeks) of control in the one and two hour ischemia groups, respectively. The same results for SM were 60 % (1 day), 42 % (week) and 61 % (2 weeks) following 1 hour ischemia. Tetanic isometric force measurements after one hour of ischemia followed by 2 and 24 hours of reperfusion are suitable to study the effect of different therapeutic modalities.

POTENTIATION OF DECREASED PYRUVATE DEHYDROGENASE ACTIVITY BY INFLAMMATORY STIMULI IN SEPSIS. 277

L. Martin, D. Remick* and T. Vary.
Penn State University, Hershey, PA 17033 and University of Michigan, Ann Arbor, MI 48109

Pyruvate dehydrogenase complex (PDH) inhibition in skeletal muscle has been suggested to contribute to the hyperlactatemia observed in sepsis. To further characterize the factors leading to this inhibition, PDH activity was measured in skeletal muscle extracts from animals with or without a sterile intravascular focus of inflammation (PE50 catheter) and with or without a nonlethal subcutaneous abscess. Sepsis (S) was induced by subcutaneous injection of a mixture of S. aureus, E. coli and B. fragilis into previously implanted sterile gauze sponges. Plasma tumor necrosis levels were examined 1 hour after inoculation of the sponges. Hindlimb muscle, blood for culture and WBC counts were taken 14 days following infection of the sponge.

Group	Bacteria	Catheter	% TNF Present	Counts	Bacteremia	% PDH (Active)
1. Control	-	-	0	13±1	0	30±3[a]
2. Abscess	+	-	67*	25±2	33%*	17±0.5[b]
3. Abscess	-	+	0	17±1	0	24±3[c]
4. Abscess	+	+	80*	17±1	80%*	6±1[d]

* p<0.05 vs1; a. p<0.001, 1vs4; b. p<0.005, 1vs2; c. p<0.001, 3vs4; d. p<0.05, 2vs4

The results show that inflammation and nonlethal infection can produce changes in PDH activity in skeletal muscle to the same degree as observed in intraabdominal sepsis. The decreased PDH activity appears to be related to the presence of a bacterial focus. The magnitude of the inhibition is dependent on the amount of inflammation as the effect is potentiated by the placement of catheters in the intravascular space.

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278 THE EFFECT OF DICHLOROACETATE ON LIPOPOLY-SACCHARIDE-INDUCED LIVER INJURY IN D-GALACTOSAMINE-SENSITIZED STARVED RATS.

K. Irita, H. Okabe, A. Koga*, M. Yamakawa, J. Yoshitake Dep. Anesth. Crit. Care Med., Dep. Surgery*, Kyushu Univ. Sch. Med., Fukuoka 812, Japan

Dichloroacetate (DCA) is known to increase pyruvate dehydrogenase (PDH) activity and to promote ketogenesis. We found previously that DCA lowered liver injury induced by CCl_4 both in starved and in unstarved rats. In the present paper, we investigate the effect of DCA on lipopolysaccharide(LPS)-induced liver injury in D-galactosamine (D-gal)-sensitized starved rats. **Methods**: LPS from E. coli, 10 μg and D-gal, 40 mg were injected i.p. into male Wistar rats (200g) starved for 24 hr. DCA 50 mg was administered i.p. 30 min later. The rats were kept starved, and blood samples and the livers were taken 24 hr after the LPS+D-gal injection. **Results**: DCA lessened the increase in serum ALT activity (668±1167 vs 1915±2903 U/L). Histological damage was reduced in DCA-treated rats. DCA lessened the increase in serum pyruvate levels induced by LPS+D-gal. DCA also counteracted the liver injury-associated inhibition of starvation ketosis.
Conclusion: DCA was shown to lessen endotoxin-induced liver injury and metabolic disturbances in starved rats. Whether the beneficial effect of DCA is due to its increasing PDH activity, due to promoting ketogenesis, or due to some other mechanism should be elucidated.

279 ENERGY CHARGING OF SEPTIC KIDNEYS WITH INTERMEDIARY PRODUCTS OF GLYCOLYSIS

K.Kürten, Ferdinand-Sauerbruch-Hospital, Dep.of Surg., FRG;

It was the aim of our investigation, to discover the changes of kidney function and energy metabolism caused by intravenous injection of 2mg/kg E. coli endotoxin and to compensate the metabolic disorders by energy charging with intermediary products of glycolysis. Methods: Narcotized mongrel dogs were provided with catheters placed into each arteria renalis; also ureters were canulated. Then septic shock was induced by i.v. injection of 2mg/kg E.coli endotoxin. Intrarenal infusion of glucose-insulin-potassium(GIK), glucose-6-phosphat(G6P), fructose-1,6-diphosphat(FDP) or lactate was performed parallel to the developing shock. During 2 hrs. the PAH-clearances were measured, then the kidneys were removed and the intracellular content of ATP, ADP, AMP, glucose, pyruvate and lactate was determined. The energy charge(EC) and total adeninenucleotide content(TAN) were calculated.

Resultes:	PAH*	TAN+		PAH*	TAN+
sham-op	191.16	2.7	G6P	43.4	2.3
2mg/kg endotoxin	12.55	1.7	FDP	17.3	2.0
GIK	137.7	2.5	lact.	5.5	1.8

*=ml/min/100g kidney weight / +=umol/g wet weight / all groups n=6

Conclusions: 1.) Reduced kidney function during experimental septicaemia can be compensated in some extend by the apply of metabolizable energy rich compounds. 2.) A close correlation between the amount of applied energy and remaining kidney function could be found. 3.) Because of its high energetic capacity and its good cellular wall penetration in combination with insulin the GIK solution showed the very best functionary and metabolic resultes in our experiments.

280 ENDOTOXIN-INDUCED INCREASES IN REGIONAL GLUCOSE UTILIZATION BY SMALL INTESTINE: A TNF-INDEPENDENT EFFECT.

C.H. Lang, J-C A. Obih, G.J. Bagby AND J.J. Spitzer. Louisiana State Univ. Med. Ctr., New Orleans, LA 70112 USA.

Previous work by our laboratory indicates that *in vivo* glucose uptake by the ileum was elevated following the injection of lipopolysaccharide (LPS). The purpose of the present study was to determine whether this LPS-induced increase in glucose uptake was a generalized response along the length of the entire gastrointestinal (GI) tract, and to assess the relative contributions of the mucosa and muscularis to the enhanced uptake. In addition, the potential roles of tumor necrosis factor (TNF) and blood flow in the metabolic response of the intestine to LPS were examined. The *in vivo* glucose metabolic rate (Rg), which indicates tissue glucose uptake, was determined for various segments of the GI tract under basal postabsorptive conditions and 3 h after the intravenous injection of *Escherichia coli* LPS (100 μg/100 g, LD_{10} at 24 h) using the tracer 2-deoxyglucose technique. At this time, LPS-treated rats were euglycemic and intestinal Rg was elevated in all sections of the GI tract from stomach to colon (52-96%). In control animals, mucosal Rg accounted for 79% of the glucose uptake by the entire small intestine; LPS increased Rg in both the mucosa and muscularis and did not alter the fractional glucose uptake. A separate group of animals were injected intraperitoneally 2 h prior to the administration of LPS with a goat anti-murine TNF IgG antibody (20 mg) which neutralized rat TNF. The LPS-induced increase in intestinal Rg was not attenuated by the TNF antibody. In a third group of rats, cardiac output (CO) and intestinal blood flow were assessed by the injection of radiolabeled microspheres. Three hours after LPS, CO and blood flow to the small intestine were not different from control values. A consistent elevation (120%) in blood flow to the muscularis was seen in all sections of the small intestine from LPS-treated rats, while mucosal flow was only elevated in the jejunum of endotoxic animals. These results indicate that 3 h after a low dose of LPS, *in vivo* glucose uptake by the entire length of the GI tract was elevated, and that the majority of the increase was due to an enhanced uptake by the mucosa which

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was independent of blood flow changes. Furthermore, the LPS-induced increase in intestinal Rg does not appear to be dependent on elevations in plasma glucose, insulin or TNF levels. However, a consistent increase in blood flow to the muscularis of the small intestine was associated with elevated Rg in that region. (Supported by NIH GM 32654).

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FASTING ALTERS SUCKLING RAT SENSITIVITY TO LIPOPOLYSACCHARIDE (LPS) BY PRESERVING GLUCONEOGENESIS. Masakatsu Goto, W. Patrick Zeller, Michael E. Gottschalk*, and R. Morrison Hurley. Dept. Pediatr., Loyola Univ. of Chicago, Maywood, IL 60153 USA

The newborn is susceptible to endotoxic shock. Hypoglycemia is a common and life-threatening sign in newborn endotoxic shock. Fasted 10 day old rats were more resistant to LPS than fed 10 day old rats. However, the mechanism has not been well understood. Materials and Methods: 10 day old Sprague-Dawley rats were grouped and received an intraperitoneal injection of saline or S. enteritidis LPS as follows: Group 1: fed+saline; Group 2: fed+LPS; Group 3: 24 hour fasted+saline; and Group 4: 24 hour fasted+LPS. Plasma glucose changes were observed for 6 hours and 24 hour mortality was counted. The liver was isolated 4 hours after the injection in Groups 1 and 2, and 6 hours after the injection in Groups 3 and 4. Gluconeogenesis was evaluated with a liver perfusion technique. Phosphoenolpyruvate carboxykinase (PEPCK) activity, a key enzyme of gluconeogenesis, was measured.
Results: 24 hour mortality was 90% in Group 2 and 38% in Group 4 ($p<0.005$ vs. Group 2). While hypoglycemia was induced at 4 hours in Group 2 ($p<0.01$), hypoglycemia was not observed in Group 4. Gluconeogenesis and PEPCK activity decreased in Group 2, but were preserved in Group 4.
Summary: 24 hour fasting increased liver PEPCK activity and gluconeogenesis in 10 day old rats. The fast attenuated the LPS effects on mortality in endotoxic shock. The protective mechanism of the fast appears to be the preservation of liver PEPCK activity and gluconeogenesis.

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EFFECTS OF PROTEIN KINASE C MODULATION ON HEPATIC HEMODYNAMICS AND GLUCOREGULATION. J. W. Lee* and J. Filkins. Loyola Univ. Shock-Trauma Institute, Maywood, IL 60153

Protein kinase C (PKC) activation plays a key role in various hepatocellular alterations during endotoxicosis. This study evaluated the effects of PKC activation using phorbol 12-myristate 13-acetate (PMA) and PKC inhibition using the isoquinolinesulfonamide derivative H-7 on hemodynamics and glucoregulation in the isolated perfused rat liver. Livers were isolated from fed male Holtzman rats and perfused with Krebs Ringer bicarbonate solution under a constant flow of 50 ml/min at 35°C. Portal vein pressures, glucose and lactate concentrations in the medium, and oxygen consumption rates were continuously monitored by a Grass polygraph, YSI glucose and lactate monitors, and a YSI oxygen monitor respectively. PMA at concentration of 2 to 200 nM increased the portal vein pressure, increased glucose and lactate production, and decreased oxygen consumption rate in a dose-dependent fashion. H-7 (200 μM) attenuated PMA (50 nM)-induced vasoconstriction (15.1 ± 1.36 vs 10.56 ± 1.17 mmHg), glucose production rate (91.3 ± 6.15 vs 71.8 ± 2.50 μmoles/gm/hr) lactate production rate (72.4 ± 6.82 vs 53.6 ± 4.82 μmoles/gm/hr) and oxygen consumption rate ($33.\pm1.41$ vs 27.9 ± 1.75 μl/gm/min). The effects of PMA were blocked either by addition of verapamil (9 μM) or perfusion with Ca^{++}- free KRB. It is suggested that the hepatic vasoconstriction and glucoregulatory changes produced by PMA are mediated by protein kinase C activation and require Ca^{++} influx from the extracellular fluid. (Supported by NIH Grant HL 31163)

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CHANGES IN THE COMPOSITION OF PLASMA LIPOPROTEINS DURING GRAM-NEGATIVE SEPSIS IN THE RAT. S. Lanza-Jacoby, A. Tabares,* S.H. Wong and D. Baer.
Jefferson Medical College and Medical College of Pennsylvania, Philadelphia, PA 19107.

Hyperlipidemia is associated with gram-negative sepsis. In this study we characterized plasma lipoproteins of fasted and fed septic and control rats with respect to their lipid and apoprotein composition. Sepsis was induced by i.v. injection of 8×10^7 live E. coli colonies/100g body wt. Food was removed from fasted control and E. coli-treated rats after injection. Fed rats were infused intragastrically with a nutritionally complete diet for 5 days prior to E. coli treatment. Twenty-four hours later the concentration of VLDL was over 2 fold higher in the fasted E. coli-treated rats than those of the fasted control rats. All of the plasma lipoproteins from the fasted E. coli-treated rats contained significantly more lipids than the lipoproteins from the control rats. During sepsis a significantly greater percentage of apo B-100 was found in the VLDL and IDL and apo B-48 in LDL. These increases in apo B were accompanied by a significant decrease in percentage of apo E in VLDL and IDL and the percentage of apo C in LDL from the E. coli-treated rats. Feeding during sepsis led to 78% reduction in VLDL total lipids, a 49% and 89% increase in IDL and LDL total lipids, respectively. The plasma concentrations of VLDL and LDL were reduced by 43% and 55%, respectively while IDL concentration increased over 5 fold in fed E. coli-treated rats in comparison to those of the fed control rats. Apo B-48 and apo B-100 percentages increased in VLDL and LDL and apo B-100 increased in IDL from fed E. coli-treated rats; whereas apo E decreased in these fractions. Gram-negative sepsis leads to marked changes in the plasma lipoprotein composition which may be attributed to defect in peripheral hydrolysis, increased hepatic synthesis or altered hepatic uptake of VLDL remnants.

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284 CARNITIN AND LIPID METABOLISM IN POST-AGGRESSION-SYNDROME AND SHOCK.
W. Heller
Univ. of Tübingen, Dept. of Cardiovascular Surgery, Calwerstr. 7, D-7400 Tübingen, FRG

As a consequence of the operative intervention changes in the metabolism occur: they can be described as post aggression syndrome. The present study dealed with the reaction of the carnitin metabolism in the post operative state. Further, it was interesting to observe how the giving of carnitin influences the postoperative carnitin metabolism and to determine whether the postoperative energy metabolism can be thereby modified in a positive way.
25 male patients with a healthy metabolism and who had to be submitted to surgical intervention took part in the investigation. They were examined over a period of five days and subdivided into two groups: a placebo group and a test group. The postoperative trauma leads to an intensified strain of the carnitin metabolism. The energy metabolism reacts according to the post aggression syndrome. The giving of carnitin leads to a clear reaction in the carnitin metabolism. In the carnitin collective the energy metabolism shows reactions in different parts: an improved energy supply and providing of endogene energy carriers. The placebo group showed the typical reaction to the operative trauma, that is a post aggression syndrome. The importance of the lipid metabolism in the energy supply becomes obvious; the protein metabolism became increasingly catabolic. No acute lack of carnitin was to be noted; the carnitin metabolism was reduced.

285 See page 169.

Plenary Session: Therapeutic Interventions—Sepsis and Organ Failure

286 THERAPEUTIC INTERVENTIONS - SEPSIS AND ORGAN FAILURE
SURGICAL APPROACH
O. Trentz and H.P. Friedl, Department Chirurgie, Klinik für Unfallchirurgie, Universitätsspital, CH-8091 Zürich, Switzerland

Multiple organ failure (MOF) and sepsis are the predominant cause of late mortality after trauma. Our current clinical approach emphasizes the simultaneous actions of

- Resuscitation and
- Source Control followed by
- Appropriate Nutrition and
- Metabolic Support.

Namely with early surgical intervention reductions in the incidence and mortality of MOF have been observed. Concerning our surgical approach several aspects should be emphasized:

- Control of Blood Loss
- Debridement of Necrotic Tissues
- Early Fracture Stabilization
- Control of Potential Septic Sources

The current presentation will specifically address these issues and will review our clinical experience with multiply injured patients susceptible to the development of posttraumatic sepsis and multiorgan failure post trauma.

287 THERAPEUTIC INTERVENTIONS: SEPSIS AND ORGAN FAILURE. R.C. Bone, Rush-Presbyterian-St. Luke's Medical Center, Department of Internal Medicine, Chicago, Illinois, U.S.A.

While many advances have occurred in our understanding of the pathogenic mechanisms and treatment of septic shock, our ability to rescue a patient from it has, unfortunately, lagged behind. Its definition is simply the deranged metabolic state that arises from systemic sepsis in a hypotensive patient unresponsive to fluid management. Septic shock may be precipitated by a variety of organisms. Invasion into the bloodstream by bacteria or their toxic metabolites triggers various pathophysiologic sequelae that eventually lead to hypotension and multiple organ failure. The syndrome of multiple organ failure is defined as the presence of two or more organ systems with impaired function. The syndrome is characterized by a hyperdynamic, hypermetabolic state identical to that seen in the septic syndrome (hypothermia(temperature <96°F rectal) or hyperthermia (temperature >101°F rectal), tachycardia (>90 beats/minute), tachypnea (>20 breaths/minute), a presumed site of infection, and evidence of inadequate perfusion (as evidenced by either poor or altered cerebral function), arterial hypoxia (PaO_2<75 mm Hg), and elevated plasma lactate level or urine output (<0.5 ml/Kg body weight/hour). The mortality rate

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associated with multiple organ failure exceeds 60%. One way of organizing a treatment strategy is to divide the approaches into the following three groups: 1) Essential therapies (e.g. antibiotics, fluids, oxygen, cardiotropic agents); 2) Controversial therapies (e.g., steroids, heparin, naloxone, cyclo-oxygenase inhibitors); and 3) Future therapies (e.g. monoclonal antibodies to endotoxin or tumor necrosis factor, passive immunization for bacterial antigens and toxins, platelet activating factor antagonists, interleukin-1 receptor antagonists). This presentation will focus primarily on these new therapies.

PREVENTION OF MULTIPLE ORGAN FAILURE BY SDD. C.P. Stoutenbeek, Dept. Intensive Care OLVG Hospital, 1e Oosterparkstraat 179, 1091 HA Amsterdam, Holland

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Multiple organ system failure (MOF) is a major problem in critically ill patients. Although it is widely recognized that MOF may be associated with uncontrolled infection, frequently no evidence of an infectious focus is present. The gut is a central organ in the pathogenesis of MOF. Translocation of variable bacteria from the gut through the mucosal barrier and the absorption of gut-endotoxin plays a crucial role in the development of MOF and sepsis syndrome. Many investigators have focused on the permeability changes of the gut mucosa by ischemia, trauma, burns, sepsis or by protein-malnutrition. However, the composition of the microflora seems to be even important. In the critically ill patient the oropharyngeal and gastro-intestinal flora changes very rapidly: the concentration of aerobic GNB and other potentially pathogenic microorganisms increases dramatically; sites that are normally almost sterile (e.g. the small intestines or the stomach), are colonized by pathogens; and colonization by (multiply resistant) microorganisms with a high intrinsic pathogenicity occurs.

Aerobic gram-negative bacilli (GNB) are predominantly responsible for translocation and endotoxin. Aerobic GNB translocate more readily than enterococci, whereas anaerobes translocate rarely. Moreover, the aerobic GNB are a larger source of endotoxin than the anaerobes. Colonization of the stomach or small intestines may also play a role because the small intestine is less resistant to translocation than the colon.

Selective decontamination of the digestive tract (SDD) prevents overgrowth of the oropharynx and GI tract by hospital-acquired potentially pathogenic microorganisms. SDD has been shown to reduce the endotoxin load in the gut by more than 90%. SDD seems to be an effective tool to prevent MOF and the sepsis syndrome in the critically ill patient.

IMBALANCE BETWEEN OXYGEN DEMAND AND OXYGEN SUPPLY.

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Jean-Louis Vincent, MD, PhD. Free University of Brussels, Belgium

Circulatory shock is defined by an imbalance between the oxygen needs of the tissues and the oxygen supply to them. Septic shock is characterized by three essential factors: First, oxygen demand is increased in relation to the inflammatory process. Second, the oxygen extraction capabilities of the cells are limited primarily by the complex peripheral alterations so that the oxygen consumption strongly depends on oxygen transport. Third, the myocardial contractility is depressed early in the course of severe sepsis and this can account for an inadequacy of oxygen delivery despite a relatively normal or high cardiac output. Among other factors, the release of the various mediators of sepsis have been implicated in the three phenomenons.

Fluid therapy is often not sufficient to correct these hemodynamic disturbances. Adrenergic therapy remains the mainstay of pharmacological therapy. Vasopressors like dopamine and even norepinephrine can be initially indicated to restore a minimal tissue perfusion pressure. Unfortunately, the effects of these substances on the oxygen extraction defect associated with sepsis are rather limited. Inotropic therapy with dobutamine can be indicated to increase oxygen transport by increasing myocardial contractility. The place of inodilators like dopexamine or phosphodiesterase inhibitors is limited. Dopexamine can increase mesenteric and renal blood flow without vasoconstrictive effect. The association of phosphodiesterase inhibitors with adrenergic agents might be considered in presence of severe heart failure.

Finally, monoclonal antibodies directed against endotoxin or tumor necrosis factor may have significant effects on both the cardiac function and the peripheral circulation.

THE USE OF ANTIENDOTOXIN MONOCLONAL ANTIBODY TO TREAT GRAM-NEGATIVE BACTEREMIC SHOCK.

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Elizabeth J. Ziegler, Department of Medicine, University of California San Diego, UCSD Medical Center, San Diego, CA 92103.

There is increasing evidence that endotoxin in the circulation is an important trigger for the cascade of events leading to organ failure and death in gram-negative bacteremia (GNB). For that reason efforts have been directed toward developing antibodies against endotoxin to try to improve the outcome in GNB. Human polyclonal antibody against common core determinants of endotoxin protects experimental animals against lethality from endotoxin and gram-negative infection and was shown in a randomized trial to prevent death in patients with GNB (New Engl. J. Med. 307:1225, 1982). To circumvent the practical

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problems of making human antiserum we sought a protective human monoclonal antibody (mAb) against endotoxin. A human IgM with such properties was developed (PNAS 82:1790, 1985) and found to be protective in animals. This mAb, called HA-1A, is directed to an epitope on lipid A, the toxic moiety of endotoxin. HA-1A has been subjected to a randomized, double-blind, placebo-controlled trial in septic patients (Clin. Res. 38:304A, 1990). Of 543 patients treated, 200 had GNB. In patients with GNB, HA-1A reduced mortality by 39% (from 49%-placebo to 30%-HA-1A, p = 0.014). Protection from HA-1A was evident in patients with shock (p = 0.017). HA-1A recipients experienced more rapid resolution of septic complications and a higher percentage were discharged alive. In a substudy Wortel *et al.* (abstract 495 Proc. Int'l. Cong. for Infect. Dis., Montreal, 1990) observed that: 1) protection with HA-1A was particularly striking in patients who had endotoxemia at base line; and 2) HA-1A treatment cause a significant decrease in serum levels of tumor necrosis factor at 24 hours (p < 0.05). All patients tolerated HA-1A well, and no anti-HA-1A antibodies were detected. Thus, human mAb HA-1A appears to be safe and effective for immunotherapy of gram-negative bacteremia and shock and its beneficial effects seem to be due to interference with endotoxin-induced activation of host defense cells.

S14: Surgical Approach

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THERAPEUTIC INTERVENTIONS - SEPSIS AND ORGAN FAILURE. O. Trentz and H.P. Friedl. Department Chirurgie, Klinik für Unfallchirurgie, Universitätsspital, CH-8091 Zürich, Switzerland

Multiple organ failure (MOF) occurs in response to infection, perfusion deficits, a persistent inflammatory focus, or a persistent focus of dead and/or injured tissue. Several aspects are considered relevant to current clinical practice. Their application in settings of trauma and surgical sepsis reduces overall mortality and incidence of multiple organ failure:

- Microsurgical Resuscitation: Since time is a critical factor in damage control, resuscitation and restoration of microvascular perfusion needs to occur as soon as possible if multiple system organ failure is to be avoided during the later time course.
- Source control: The best treatment for multiorgan failure appears to be prevention. With early, aggressive control or removal of risk factors for multiple organ failure, namely early surgical intervention for control of potential septic sources and early fracture stabilization reductions in the incidence and mortality of MOF have been observed.
- Metabolic Support: Malnutrition appears to be an important cofactor in morbidity and mortality. Metabolic support needs to be started early and prior to the phenomenon of nitrogen retention during the hypermetabolic state of multiple organ failure.

In the clinical setting other aspects may become useful in the near future:

- Drug Therapy: Drugs to antagonize oxidant injury pathways, specific inhibitors of leukotriene receptors and vasoactive prostanoids appear to have beneficial effects in the experimental setting.
- Directed Antibodies: Antibodies against various lipopolysaccharide components and different compounds of mediator cell functions are currently developed and require further research to determine future applications in clinical treatment.
- Growth Factors: Modulation of growth factors may be useful in promoting parenchyme healing without adverse fibroblasia.
- Nutritional Modulation: immune functions and protein synthesis may be altered by dietary therapy.

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SURGICAL APPROACH TO AVOID ORGAN FAILURE IN MULTITRAUMA PATIENTS. L. Schweiberer, D. Nast-Kolb, C. Waydhas, K.-G. Kanz. Ludwig-Maximilians-Universität München, Klinikum Innenstadt, Chirurgische Klinik und Poliklinik.

Our multitrauma protocol is guided by a step by step management plan as already published in 1978. If an unstable shock is present, life saving interventions and operations have to be immediately performed within minutes. By these procedures some of the fatal injured patients may be saved. To prevent late death due to multi organ failure as effects of the primary hemorrhagic and traumatic shock, additional and amplifying trauma by operations must be minimized. Consequently only life, organ or limb threatening injuries, especially fractures, are treated within days. In a prospective study we verified this management plan by biochemical findings. In more than 100 multitrauma patients a maximal release of mediators was found within the first hours, followed by a general decrease within the next two days. So the time between the second and the fourth day is the optimal time for elective surgery. Patients with following organ failure can be clearly identified by significant different higher and persisting levels of mediators. In these cases we recommend a minimal traumatising surgery, i.e. for femur fractures fixation by fixateure externe instead of intramedullary nailing.

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CAUSES OF DEATH FOLLOWING INTRA-ABDOMINAL SEPSIS: A PROSPECTIVE ANALYSIS OF 300 PATIENTS. J.L. Toth, J.M.A. Bohnen, B.D. Schouten, R.A. Mustard (Spon: W. Sibbald)

Intra-abdominal sepsis (IAS) is known to be associated with a high mortality rate although the exact cause of death amongst this group of patients remains unclear. This study examines 300 patients with intra-abdominal sepsis. Data was collected prospectively on all admissions with IAS between 1984 and 1989. Eighty-nine patients (30%) died in hospital. The cause of death of these patients could be classified into four categories: i) premorbid otherwise fatal condition ie. end stage carcinoma, ii) entirely due to sepsis ie. multi-system organ failure (MSOF) or septic shock, iii) sepsis contributing to death from exacerbation of underlying disease eg. COPD, CAD, etc., and iv) miscellaneous causes not directly related to sepsis. The following distribution of causes of death was found:

Otherwise fatal	MSOF or shock	Sepsis contributing	Misc	Total
22(25%)	36(40%)	24(27%)	7(8%)	89

Fully one-third of fatalities in patients with IAS could not have been prevented with any therapy for sepsis. This group of patients, particularly those with advanced malignancies, should be excluded from clinical trials of therapeutic modalities.

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MULTIPLE ORGAN FAILURE IN DIFFUSE PERITONITIS.

W. Barthlen, H. Bartels
Surgical Clinic, Technical University, D-8000 Munich, FRG

The incidence of multiple organ failure (MOF) secondary to diffuse peritonitis and subsequent mortality was examined in a group of 184 patients managed by staged laparotomy. Mortality was found to depend on the timing and the success of surgical toilet: early elimination of the septic focus 6%, delayed elimination 19%. Mortality was 100% if the septic focus could not be removed by surgical toilet.
The overall mortality was 26,1%. Respiratory failure occurred in all patients. Cardiac, renal, hepatic and pancreatic failure, as well as abnormalities of clotting and blood sugar control occurred in between 10 and 30% of the surviving patients (n=136). MOF was diagnosed on average 3,9 days after onset of peritonitis. The average duration was 11,8 days. MOF was diagnosed in between 42 and 75% of those patients who died in spite of successful surgical toilet (n=14). The average onset of MOF in this group was 8,2 days with an average duration of 19 days. MOF occurred in between 59 and 100% of patients who died without focus elimination being achieved (n=34). The average onset of MOF after diagnosis of peritonitis was 12 days and continued on average for 27,2 days.
Conclusion: Multiple organ failure was an infrequent problem after diffuse peritonitis in those patients who survived. When it did occur, it was clinically apparent soon after onset and rapidly reversible by early surgical toilet. In those patients who died despite aggressive treatment, MOF was judged to have been reversible in the early stages of the disease. If surgical removal of the infectious source was delayed or not possible, MOF occurred late and was not reversible.
It is recommended that in all patients with diffuse peritonitis, early aggressive surgical toilet should be performed in order to remove the septic focus.

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INFLUENCE OF ACUTE PASSIVE HEPATIC CONGESTION ON HEPATIC ENERGY STATUS IN DOGS. H. Higashiyama*, Y. Takada*, S. Iwata*, M. Yamaguchi*, K. Kumada*, H. Sasaki*, Y. Shimahara*, K Ozawa* (Spon: H. Hirasawa).

Second Dept. of Surg. Kyoto Univ. Kyoto, Japan

Temporary hepatic outflow occlusion is sometimes required in trauma or at hepatectomy. However, the tolerance limit of acute passive hepatic congestion (APHC) caused by this procedure remains to be clarified. The present study reports an APHC model in dogs by clamping the thoracic inferior vena cava with passive veno-venous shunt and investigates the effect of APHC on hepatic energy status by assessing the changes in arterial ketone body ratio (KBR), reflecting the hepatic mitochondrial redox state, and hepatic energy charge (EC). After induction of the APHC, portal vein pressure elevated almost 3 times of the preclamping level. KBR decreased significantly ($p<0.05$) for 60 min, but gradually recovered thereafter, and returned to the preclamping level after the reversal of 120 min. Although total hepatic blood flow during APHC was approximately 18% of the preclamping value, no significant differences of the EC and the liver function tests were observed between preclamping and at 120-min clamping. The liver biopsy sample taken at 120-min clamping showed marked sinusoidal congestion and lymphatic dilatation, while the hepatic parenchyma surrounding the portal area was relatively well preserved. All dogs survived at least 1 week. These results suggest that the liver could tolerate the APHC for 120 min, which would enable surgeons to perform the vascular reconstruction needed for declamping the hepatic outflow block and other radical repairs.

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298 MULTIPLE ORGAN FAILURE (MOF) AFTER OPEN HEART SURGERY. M.Nakamura, K.Tanaka, K.Tujimura, T.Izumi and K.Suekane
Dept of Anesthesiology, Kinki Univ. Sch. Med.,Osaka-sayama,Osaka,589 Japan

The retrospective study of 120 consecutive patients undergoing open heart surgery was undertaken to analyze the cause of MOF after open heart surgery.
MOF was diagnosed more than two organ failures were found. The diagnosis of low-output syndrome (LOS) was done when CI was lower than 2.21/min/m^2 .
In 120 cases, 25 patients were diagnosed as MOF and 6 of them died. The incidence of each organ failure was as follows: heart (36cases;30%), lung (19cases;16%), liver (16cases; 13%), kidney (15cases;13%) and brain (8cases;7%). 72% of MOF patients were suffered from LOS and the ratio was significantly higher than that of LOS in non-MOF patients (19%). The severe infection and DIC were included only in MOF patients (infection;24%, DIC;8%) and the prognosis was very poor.
These findings suggest that LOS and the following infection plays an important role as a cause of MOF after open heart surgery. To prevent MOF after open heart surgery, LOS and infection have to be avoided or treated as early as possible.

S15: Vasoactive Drugs—Circulatory Support

299 USE OF VASOACTIVE AGENTS IN THE MANAGEMENT OF SEPTIC SHOCK. Jean-Louis Vincent. Dept. of Intensive Care, Free University of Brussels, Belgium

Fluid therapy is the basis for therapy of septic shock. However, it is not always sufficient to correct the associated hemodynamic disturbances. Adrenergic therapy remains the mainstay of pharmacological therapy. Vasopressors like dopamine and even norepinephrine can be initially indicated to restore a minimal tissue perfusion pressure. Unfortunately, the evidence is limited that these substances can increase the oxygen extraction capabilites associated with sepsis. Inotropic therapy with dobutamine can be indicated to increase oxygen transport by increasing myocardial contractility. This form of therapy could be valuable even when cardiac output is normal or high . The place of inodilators like dopexamine or phosphodiesterase inhibitors is limited. Dopexamine can increase mesenteric and renal blood flow without vasoconstrictive effect. The association of phosphodiesterase inhibitors with adrenergic agents might be considered in presence of severe heart failure.

300 THE EFFECTS OF CATECHOLAMINES ON OXYGEN DELIVERY/CONSUMPTION AND CELLULAR INJURY IN PATIENTS WITH POSTOPERATIVE SEPTIC MULTIPLE ORGAN FAILURE(SMOF). S. Oda*, H. Hirasawa, T. Sugai*, Y. Ohtake, H. Shiga*, K. Matsuda*, and N. Kitamura*
Department of Emergency and Critical Care Medicine, Chiba University School of Medicine,Chiba, Japan

The present study was undertaken to evaluate the oxygen metabolism in SMOF and the effects of catecholamines on it. Fourteen postoperative SMOF patients (6 died, 8 survived) were assessed their oxygen delivery ($\dot{D}O2$)/consumption ($\dot{V}O2$) in relation to cellular injury score (CIS) derived from 3 different intracellular metabolic sequences, arterial ketone body ratio (AKBR), osmolality gap (OG), and blood lactate. The changes of these parameters by catecholamine administration were also studied. Ten ICU patients without organ failure were served as controls. In the early stage, SMOF group showed hyperdynamic state and increased $\dot{V}O2$, reflecting increased oxygen demand in tissue. However, CIS of SMOF was significantly higher than that of control group in spite of increased oxygen consumption, indicating that oxygen debt may exist in vital organs resulting in cellular injury. Further deterioration of intracellular metabolism expressed by increase in CIS was accompanied by decreased $\dot{D}O2$ and $\dot{V}O2$. Although catecholamines could increase $\dot{D}O2$, $\dot{V}O2$ and CIS were not effectively improved by catecholamine administration, especially among the patients with severe cellular damage. These data suggest that appropriate catecholamine administration should be considered earlier to improve oxygen metabolism in SMOF patients.

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THE EFFECTS OF NOREPINEPHRINE ON HEMODYNAMICS AND RENAL FUNCTION IN SEVERE SEPTIC SHOCK STATES. E.M.Redl-Wenzl, C.Armbruster, G.Edelmann, E.Fischl, M.Kolacny, A.Wechsler-Fördös and P.Sporn (Spon: G.Schlag). Dept. of Anesthesiology and 1st Dept. of Surgery; KA Rudolfstiftung, 1030 Vienna, Austria

In extreme high output-low resistance states adequate perfusion pressures cannot be maintained despite extreme increases of cardiac output. We investigated the impact of norepinephrine (NE) in 56 patients in severe septic shock states (36 diffuse peritonitis, 14 superinfected nectrotizing pancreatitis, 6 miscellaneous septic cases). Study enter criteria: MAP<60 torr despite volume optimization and dopamine (DP) >20mcg/kg/min or DP+dobutamine>30mcg/kg/min. After registration of baseline values reduction of DP to 2.5mcg/kg/min and start with NE at a dose rate of 0.05 mcg/kg/min until MAP>60torr. Statistical evaluation: Analysis of variance and t-test (*p<0.01, **p<0.001). Results: 31 patients survived, 25 died (44.6%). NE dosage: 0.1-2 mcg/kg/min (mean: 0.4 mcg/kg/min).

	Control	1hNE	24hNE	48hNE
MAP (torr)	56±4	77±11**	82±8**	
CI (l/min)	3.8±1.1	3.7±1.2	4.1±0.8	
WP (torr)	9±4	9±3	9±3	
TPRI (dyne.s/cm5)	1023±286	1573±539**	1512±369**	
O2AVI (ml/min)	566±170	552±175	617±159	
VO2I (ml/min)	160±57	164±62	171±69	
CCr (ml/min)	75±37		89±39	102±43*

Conclusion: In extreme low resistance states particular attention has to be paid to sufficient mean arterial pressures. Our results suggest that this essential goal can be achieved by NE. MAP and glomerular filtration rate improved markedly without deleterious effects on CI, oxygen delivery and oxygen consumption.

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ENHANCEMENT OF URINE OUTPUT AND GLOMERULAR FILTRATION IN ACUTELY OLIGURIC PATIENTS USING LOW DOSE NOREPINEPHRINE J. Cesare, J. Ligas, E. Hirvela
St. Francis Hospital & Medical Center, Hartford, CT

It has been observed that urine output is frequently improved in septic patients after initiation of norephinephrine therapy, sometimes without alteration of hemodynamic parameters. Norepinephrine in low doses has been demonstrated, in animals, to have selective vasoconstrictive action on the efferent *glomerulus, resulting in increased* filtration and urine output. To test the hypothesis that low dose norepinephrine enhances urine output and renal function in oliguric patients, a prospective clinical trial was undertaken. Under strict monitoring, protocol guidelines, and human use approval, norepinephrine (0.05 and 0.1ug/kg/min) was instilled in oliguric (≤ 0.5 ml/kg.hr), volume replete, hemodynamically stable patients following a control collection period. Urine output and creatinine clearance were measured as a reflection of renal function. Hemodynamic parameters, including heart rate, systolic, diastolic, and mean blood pressure, cardiac output, pulmonary capillary wedge pressure and systemic vascular resistance were recorded for each patient.

Results:	Control	Study		
HR	101	99	p=0.75	
MAP	83	94	p=0.004**	
CO	7.4	7.3	p=0.82	n=9 **statistically different
SVR	846	930	p=0.24	
CrCl	67.5	99.2	p=0.002**	
Urine/H	29.1	43.2	p=0.009**	

There was an average increase of urine output of 13 ml/hr. This was accompanied by an increase in creatinine clearance of 31.7 ml/min. The only hemodynamic parameter which varied significantly was mean arterial pressure, which increased 10mmhg. We conclude that infusion of norepinephrine at extremely low doses may be of significant benefit in augmenting renal function and urine output in acutely oliguric patients.

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OXYGEN DELIVERY AND UPTAKE IN CIRRHOSIS: EFFECT OF VASOPRESSORS
K.Lenz, H.Hörtnagl, W.Druml, A.N.Laggner, G.Grimm, B.Schneeweiß Intensive Care Unit Vienna

By infusing positive inotrope and vasodilating substances oxygen consumption (VO2) in cirrhotic patients was shown to be dependent on oxygen supply possibley due to an inadaquate tissue oxygen extraction (O2Extr.). However a direct effect of vasoactive agents on VO2 may have contributed to altered VO2. Thus, we investigated DO2 and VO2 during infusion of a non-catecholamine vasopressor in decompensated cirrhosis patients.
Patients and methods: 9 patients (7male, 2female, mean age 47±7years) with decompensated cirrhosis (Child C) were studied. Hemodynamic monitoring was performed by a flow directed pulmonary arterie catheter. Hemodynamic parameters, arterial and venous oxygen concentration of norepinephrine (NA) and adrenalin (A) were evaluated before and 2 hours after starting the infusion of the vasopressor agent 8-ornipressin (POR 8, 6U/h)

Results:	CO (L/min)	O2 Extr %	DO2 ml/min.m²	VO2ml	NA (ng/ml)	A (ng/ml)
before POR	12.5± 0.7	19.8±1.7	707 ± 4o	136 ± 8	1.35 ± 0.19	0.74 ± 0.5
during POR	9.0± 0.6⁺	26.1±2⁺	538 ± 3o⁺	133 ± 6	0.6 ± 0.22⁺	0.48 ± 0.3⁺

Values are mean + SEM ⁺p 0.05
Conclusion: In patients with decompensated cirrhois no denpendency of VO2 on DO2 was seen during the infusion POR8. The decrease in DO2 caused by a decrease in CO could be effectevely compensated by an increase in O2 Extr. Nevertheless htere was a decrease in sympathe-

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tic outflow indicating a decrease in oxygen demand. Thus, unchanged oxygen consumption indicates either an insufficient oxygen supply in cirrhotics before vasopressor infusion and/or a shunting of blood from regions with low oxygen extraction to regions with high oxygen extraction

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DIGITAL ISCHAEMIA IN THE INTENSIVE CARE UNIT. M. Hayes*, E. Yau* and D. Watson* (Spon : R. Little).
St. Bartholomew's Hospital, West Smithfield, London, EC1A, England.

Noradrenaline is once again being widely advocated for the management of septic shock on the basis of the observed low systemic vascular resistance seen in this condition. We have encountered three patients treated with noradrenaline during hyperdynamic sepsis who developed marked digital ischaemia which may have been caused or exacerbated by such therapy. Case 1 - a 29 year old male who developed septic shock following urinary tract surgery. Blood cultures yielded Streptococcus faecalis. Antibiotics were commenced and his hypotension was treated with a noradrenaline infusion in view of a low systemic vascular resistance index (SVRI)$_5$. He developed ischaemic toes on day 7. The maximum derived SVRI was 617 dyne/sec/cm^5.m^2. He ultimately died from multiple organ failure on day 10. Case 2 - a 44 year old male admitted with severe pneumonia. Blood cultures yielded type B Haemophilus influenzae. Antibiotics were commenced and treatment of his hypotension was with a combination of noradrenaline and dobutamine. He developed ischaemic toes on day 3. His maximum derived SVRI was 988 dyne.sec/cm^5.m^2. He died from multiple organ failure on day 11. Case 3 - a 35 year old woman admitted with bleeding oesophageal varices requiring massive transfusion and intravenous vasopressin. On day 4 both her fingers and toes were noted to be cold and dusky. On the same day she became pyrexial and hypotensive. Blood cultures later yielded Escherichia coli. She was commenced on antibiotics and her hypotension was treated with low dose noradrenaline. Her SVRI at this time was 1100 dyne.sec/cm^5.m^2. This patient survived, however the ischaemia of her toes proved irreversible. Calculation of SVRI following measurement of cardiac output clearly cannot reflect intensive vasoconstriction in a localised vascular bed. Meticulous clinical monitoring is imperative since inadvertent digital ischaemia may still occur during noradrenaline administration.

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DOBUTAMINE AND NOREPINEPHRINE ADMINISTRATION IN A DOG MODEL OF ENDOTOXIC SHOCK.

Jan Bakker* and Jean-Louis Vincent.
Department of Intensive Care, Erasme University Hospital, Route de Lennik 808, B-1070 Brussels, Belgium.

In septic shock the effects of adrenergic agents on oxygen delivery($\dot{D}O_2$) and oxygen consumption($\dot{V}O_2$) remain controversial. We compared the effects of dobutamine(DOB) and norepinephrine(NE) administration on hemodynamics, $\dot{D}O_2$ and $\dot{V}O_2$ during endotoxic shock in dogs. The study included 14 dogs(22 ± 1 kg) pentobarbital anesthetized and mechanically ventilated with air. Thirty min after the injection of 2 mg/kg of E. coli endotoxin, fluid challenge using 0.9% saline was performed to restore and maintain filling pressures at baseline. The protocol was randomized for agent(DOB/NE) and dose(Hi/Lo). In the first 7 dogs DOB 5(Lo) and 10 ug/kg.min(Hi) and NE 0.1 and 0.2(Lo) ug/kg.min were used. In the other 7 dogs Lo- and Hi-DOB and NE 0.5 and 1.0 ug/kg.min(Hi) was used. Each agent was infused for 20 min at each dose with a drug-free interval. Data were analyzed by Student's t-test for paired data(mean±SEM). Both DOB and Hi-NE increased heart rate and cardiac output(CO) significantly, whereas mean arterial pressure(MAP) was increased by both Lo- and Hi-DOB(from 85±7to 96±7, p<0.001 and from 78±5 to 94±7 mmHg,p<0.01) and NE (from 87±3 to 100±5,p<0.05 and from 82±6 to 99±7 mmHg,p<0.001). Similarly, Lo- and Hi-DOB significantly increased $\dot{D}O_2$(from 891±91 to 1142±99,p<0.001 and from 847±109 to 1317±75 ml/min,p<0.001) whereas only Hi-NE increased $\dot{D}O_2$ (from 969±62 to 1240±49 ml/min,p<0.001). The extraction ratio(ER) decreased significantly with Lo- and Hi-DOB (from 21.6±1.6 to 16.9±1.1,p<0.05 and from 21.1±1.8 to 16.0±1.2%,p<0.001) but not during Hi-NE. ER was more reduced with DOB then with Lo-NE(p<.05). In this endotoxic shock model, Lo-NE increases MAP but does not consistently increase $\dot{D}O_2$ and $\dot{V}O_2$. High dose NE preserves ER better than DOB, but DOB produces a more consistant increase in $\dot{D}O_2$ and $\dot{V}O_2$.

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THE ROLE OF VASOACTIVE DRUGS IN THE THERAPY OF SEPTIC SHOCK. K. Reinhart, L. Hannemann. Dep. of Anesthesia and Intensive Care Medicine, Klinikum Steglitz, Free University of Berlin.

Inadequate tissue oxygenation plays a major role in the pathogenesis of multiple systems organ failure (MOF). In septic pathologic cellular O2 supply involves the impairment of convective O2 delivery (DO2) to the tissue, as well as the regional microcirculatory blood flow. Vasoactive drugs for hemodynamic support in septic shock should not be judged primarily by their effects on systemic blood pressure but the specific effects of treatment on regional and nutritive blood flow could be especially taken into account. The most promising and ideal drugs are those that not only increase global DO_2 to adequate levels, but also

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counteract the pathologic impairment of regional and microcirculatory blood flow by directing tissue DO_2 to areas where it is needed most. It has been demonstrated that by achieving supranormal levels of DO_2 tissue oxygenation can be improved. If the cause of inadequate DO_2 is considered to be a low or inadequate cardiac output, despite adequate volume loading inotropic support is necessary. In severe cases a vasopressor is additionally warranted to achieve adequate organ perfusion pressures.

PROSTAGLANDIN E_1 LOWERS CRITICAL OXYGEN DELIVERY (DO_{2crit}) IN NORMAL PIGS. A.B.J. Groeneveld*, C. Vermeij*, Thijs L.G.
Medical Intensive Care Unit, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

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The DO_{2crit} may be elevated in septic shock, partly caused by blood flow maldistribution, and vasodilating prostaglandins may be involved. However, these substances may improve tissue oxygenation, perhaps through reduced capillary microembolization and a rise in the surface area available for O_2. We therefore studied if infusion of vasodilating prostaglandin E_1 (PGE_1, 0.2 $\mu g.kg^{-1}.min^{-1}$) alters the DO_{2crit} in normal, barbiturate-anesthetized pigs (n=12, ±22 kg). Using a cross-over design, we infused PGE_1, followed or preceded by saline, for 90 min. We lowered cardiac output by incremental positive end-expiratory pressure (PEEP, 0-20 cm H_2O, in steps of 5 cm H_2O at 15 min. intervals). We measured pressures, blood flow (thermodilution), O_2 uptake (VO_2, metabolic monitor), blood gases and lactate before, during and after these interventions. We calculated DO_2. During saline infusion, DO_2 fell by 60±7% (mean±sd) and VO_2 by 28±7% from baseline to 20 cm H_2O PEEP. The fall in DO_2 (and arterial blood pressure) during PGE_1 was larger at equivalent VO_2, but the rise in lactate was greater. Using an exponential model ($VO_2 = a*(1-e^{-b*DO_2})$), to which the data fitted well by non-linear regression analyses, DO_{2crit} at VO_2=0.9*a was 13.3±2.7 $ml.min^{-1}.kg^{-1}$ during saline and 10.5±1.5 during PGE_1 ($p<0.02$). The drug elevated the critical O_2 extraction ratio in all animals. Hence, PGE_1-induced vasodilation reduces DO_{2crit} and enhances the O_2 extraction ability in normal pigs, in spite of more severe hypotension and unchanged basal O_2 needs. The balance of distribution of blood flow according to demands and the capillary surface area available for O_2 exchange is favorably affected. A greater rise in lactate could be caused by greater production in tissues with a minor contribution to total VO_2 (i.e. skin). Vasodilating prostaglandins may not play a role in elevating the DO_{2crit} in septic shock and may be useful in the treatment of the syndrome.

MODE OF CARDIOVASCULAR IMPROVEMENT BY NALOXONE: ENHANCEMENT OF β-ADRENERGIC EFFECTS. **Adam J. Dziki*, William H. Lynch*, and William R. Law. Casualty Care Res. Dept., Nav. Med. Res. Inst., Mail Stop 15, Bethesda, MD 20889-5055 & Dept. Surg., Georgetown Univ., Wash., DC.**

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The mechanisms behind cardiovascular improvements by opioid receptor antagonists in septic shock are not understood. Enhancement of adrenergic effects by opioid receptor antagonists has been suggested. This study was designed to determine the role of the β-adrenergic influences in the cardiovascular response to naloxone treatment of endotoxic shock. Anesthetized, male, Sprague-Dawley rats (350-500g) were catheterized (tail artery, external jugular, and left ventricle via the right carotid artery). 24 hours later, endotoxin shock was induced in conscious rats by infusing E. coli endotoxin (E; 2 mg/kg in 0.5 ml) over 30 min. Next, naloxone (N; 4mg/kg bolus; 2 $mg*kg^{-1}*hr^{-1}$ infusion; 4 mg/ml solution) was administered with or without propranolol (P; 100 mg/kg bolus and 100 $mg*kg^{-1}*hr^{-1}$). Saline (S) was the vehicle control for each treatment. Mean arterial blood pressure (MAP) and heart rate (HR), were obtained with pressure transducers. Cardiac index, (CI; $ml*min^{-1}*100^{-1}gBW$) was measured using radioactive microspheres. Stroke volume index (SVI) and systemic vascular resistance index (SVRI) were calculated using standard formulas. Data (means ± SEM) analyzed with ANOVA (*sig. diff. between saline and naloxone treatment at $p \leq .05$).

Time (min)	MAP E/S/S	MAP E/N/S	HR E/S/S	HR E/N/S	CI E/S/S	CI E/N/S	SVI E/S/S	SVI E/N/S	SVRI E/S/S	SVRI E/N/S
0	116±14	134±13	435±51	422±66	29±8	32±4	67±14	79±18	346±116	327±49
60	96±15	116±19	514±93	475±102	23±7	31±4	44±11	75±20*	366±89	291±74
120	107±25	123±12	533±99	471±101	18±5	28±11*	33±11	72±8*	567±275	338±31
	E/S/P	**E/N/P**	**E/S/P**	**E/N/P**	**E/S/P**	**E/N/P**	**E/S/P**	**E/N/P**	**E/S/P**	**E/N/P**
0	113±15	111±6	385±55	362±13	24±5	30±9	64±18	82±25	391±76	344±117
60	93±19	109±3	362±53	332±34	17±8	21±9	46±18	64±28	521±172	537±328
120	101±14	111±13	336±101	373±12	19±4	20±3	59±11	54±9	485±181	444±81

Endotoxin shock alone (n=5) led to decreased CI and SVI. In the presence of intact β-adrenergic influences (no propranolol), naloxone treatment of endotoxin shock (n=4) significantly improved CI, and SVI. In the presence of β-adrenergic blockade (n=4 per group) these improvements were not seen. These results show that in endotoxin shock in rats, naloxone improves cardiac index. Addition of propranolol ablates these changes, suggesting that during endotoxin shock, naloxone induces some of its cardiovascular effects via enhancement of endogenous β-adrenergic activity.

HEMORRHAGIC HYPOTENSION AND RETRANSFUSION DEPRESSES ENDOTHELIUM-DEPENDENT RESPONSES OF FELINE MIDDLE CEREBRAL ARTERY: RESTORATION OF NORMAL RESPONSES BY L-ARGININE TREATMENT.
A.G.B. Kovách, Cs. Szabó, Cs. Csáki, Z. Benyó, G. Kiss and M. Reivich
Univ. Pennsylvania,CVRC, PA 19104-6063, USA & Exp. Res Dept, Semmelweis Med.Uviv.H-1082 Hungary

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Experiments were performed to determine whether disfunction of the cerebrovascular endothelium occurs following hemorrhagic hypotension and retransfusion. Feline middle cerebral arteries were prepared from control animals and from animals subjected to hemorrhagic hypotension (stepwise bleeding to 90, 70 and 50 mmHg, maintained for 20 min at each level) followed by retransfusion (20 min). Two mm long arterial segments were suspended in organ chambers containing Krebs solution (37°C, gassed with 95% O_2-5% CO_2) for isometric force measurement. Contractions to noradrenaline,

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endothelium-dependent relaxations to acetylcholine, ATP and adenosine and direct smooth muscle dilations to SIN-1 were compared in the vessels of the control and hemorrhage-subjected animals. The latter arteries showed enhanced contractions to noradrenaline and (except of SIN-1) impaired relaxant responses to all the relaxants tested. We investigated further the effect of exogenous application of L-arginine (L-Arg), the precursor of endothelium-derived relaxing factor (EDRF) and N^G-Nitro-L-Arginine (NOLA), a competitive antagonist of the EDRF producing enzyme on the vascular responses. In the control vessels in vitro L-Arg treatment did not modify any response. At the same time it inhibited the hemorrhagic hypotension-induced enhancement of the contractions to noradrenaline and restored the diminishment of the relaxations to acetylcholine (but not to ATP or adenosine). NOLA enhanced the noradrenaline-induced contractions and inhibited the acetylcholine-, ATP-, and adenosine-induced relaxations in the control vessels. In the vessels after hemorrhage, however, NOLA did not further potentiate the markedly enhanced noradrenaline-induced contractions, and did not further inhibit the relaxations caused by ATP and adenosine, whereas in the case of acetylcholine it caused a further (and now complete) inhibition of the relaxations. We also tested the effect of in vivo L-Arg infusion during hemorrhagic hypotension on in vitro vascular responses. The responses of these vessels were similar to those responses of the hemorrhage-subjected cats following in vitro L-Arg treatment. In conclusion, the present study demonstrates that short-lasting hemorrhagic hypotension markedly inhibits endothelium-dependent cerebrovascular responses. Normal responses to acetylcholine and noradrenaline are restored by in vivo or in vitro L-Arg treatment, suggesting a role for an exhaustion of the endogeneous L-Arg pools in the pathophysiology of the present vascular changes during hemorrhagic hypotension.

313 THERAPEUTIC EFFECTS OF DOPEXAMINE ON CARDIAC OUTPUT AND ORGAN BLOOD FLOW IN RATS DURING ENDOTOXEMIA.

A.A. van Lambalgen, M.F. Mulder, A.A. van Kraats, G.C. van den Bos, L.G. Thijs. Lab. for Physiology and Dept. of Int. Med., Free University, 1081 BT Amsterdam, The Netherlands

Endotoxin did not cause the expected low output state in rats pre- and cotreated with dopexamine (DX), a new dopaminergic and ß2-adrenergic agonist (v Lambalgen et al., J Crit Care 1991, in press). We have now tested if DX could restore cardiac output (CO) and organ blood flow when therapeutically administered during endotoxemia. Endotoxin (*E.coli*, 8mg/kg) was infused for 60 min (start at t=0) into 18 anesthetized rats; at t=60 min 8 rats received DX infusion (3.10^{-8} mol/kg.min; DX-group), the other 10 (ES-group) received saline (infusion rate 2 ml/kg.h); experiments ended at t=135 min. We measured mean arterial pressure (MAP), CO (thermodilution) and blood flow (radioactive microspheres) to heart, brain, hepatic artery, stomach, intestines, spleen, pancreas, kidneys, adrenals, diaphragm, skeletal muscle and skin at t=0 and 135 min. At t=60 CO had significantly decreased in both groups to ca 67%; it subsequently further decreased in the ES-group only (to 43%). At t=135 CO was 74 % higher ($p<.05$) in the DX- than in the ES-group; MAP behaved the same in both groups (decrease by ca 40 %). Myocardial blood flow had increased in the DX- (by 22%) and was higher than in the ES-group (by 41%, $p<.05$); skeletal muscle flow only fell in the ES-group (by 42%; difference between groups: 31%, $p<.05$); intestinal, splenic and skin blood flow also decreased in the DX- (by 40, 34 and 63%, resp.), but were still higher than in the ES-group (by 41, 81 and 142%, resp.; $p<.05$); in both groups renal, pancreatic and stomach blood flow fell (by ca 60, 70 and 60%, resp.), diaphragm blood flow had increased (by ca 30%), and adrenal, hepatic arterial and cerebral blood flow did not change. In the ES-group 4 rats died at the end of the experiment. Dopexamine, therapeutically administered during endotoxemia, thus prevented progression of the low output state; this was especially benificial for blood supply to heart, skeletal muscles, skin and intestines.

314 PLASMA CATECHOLAMINE LEVELS IN PORCINE ESCHERICHIA COLI SEPTICAEMIA AND FOLLOWING TREATMENT WITH BUPRENORPHINE AND NALOXONE.

M.D.J. Donaldson*, C.J. Vesey*, G.M. Besser*, J.D. Watson* and C.J. Hinds* (Spons : R. Little).
St. Bartholomew's Hospital, West Smithfield, London, EC1A, England.

Changes in plasma catecholamine levels were investigated in porcine E.Coli septicaemia to relate these to the severity of shock and determine the influence of naloxone or buprenorphine. Thirty farm piglets were anaesthetised with α-chloralose and infused with live E.Coli over 2 hours. 60 minutes after starting the infusion cardiac index, mean arterial pressure and pH had decreased significantly and there was a significant rise in mixed venous lactate concentrations. Animals were then randomly divided into 3 groups and received either naloxone (bolus 2 mg kg^{-1} followed by an infusion of 1.5 mg $kg^{-1}hr^{-1}$), buprenorphine (0.3 mg kg^{-1} bolus) or an equivalent volume of normal saline. Treatment with both naloxone and buprenorphine resulted in significant increases in cardiac index and a significant reduction in acidosis but no associated rise in circulating catecholamine levels. On the contrary, there was a tendency for catecholamine levels to fall in treated animals and adrenaline levels were significantly lower than controls in the buprenorphine group during the last 90 minutes following treatment and in the naloxone group 150 minutes after treatment. During the first hour of the infusion of live E.Coli median plasma concentrations of adrenaline and noradrenaline increased significantly. This was closely related to the severity of shock. In the control group, significant inverse correlations were observed between circulating catecholamines and cardiac index ($P<0.001$) mean arterial pressure ($P<.01$), pH ($P<0.001$), base excess ($P<0.001$), oxygen delivery ($P<0.001$) and oxygen consumption ($P<0.01$). A significant positive correlation was noted with mixed venous lactate. Median peak plasma catecholamine levels were significantly higher in non-survivors than in survivors ($P<0.01$) and dramatic terminal increases in catecholamine levels were observed in non-survivors.

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MECHANISMS OF LIFE-SAVING EFFECT OF PHYSOSTIGMINE IN HAEMORRHAGIC SHOCK. J.Savic, V.M. Varagic, S.Vujnov, M.Prostran and R.Spaic.
Inst. for Med. Research, Military Med. Academy, Belgrade, 11000

Physostigmine (Ph) has benn known to produce a beneficial effect in haemorrhagic shock in rats and rabbits. In this paper mechanisms of this life-saving effect were investigated. Haemorrhagic shock was produced in anaesthetized rabbits using model of intermittent bleeding of 50% of estimated (5% bw) blood volume during 30 min. An i.v. bolus of Ph (70 µg/kg) or saline (0.1 ml/kg) was injected immediately after bleeding. The results (table) show that 7 out of 11 animals in the saline (S) group died during the first hour after bleeding, while 11 out of 12 animals treated by Ph survived that period ($p<0.01$). Final survival (at 24 h) was also significantly higher in Ph group. Mean arterial pressure (MAP) in Ph treated rabbits was significantly increased compared both to postbleeding value and to S group at corresponding time intervals. Plasma volume was normalized and concentration of noradrenalin was increased. We concluded that early beneficial effect of Ph on MAP enables animal to compensate efficiently plasma loss probably by increase in sympathetic discharge.

EXP. GROUP	M A P (mmHg) and SURVIVAL (in parentheses)							
	Before bleeding	After bleeding	AFTER TREATMENT (MEAN ± SD) 5	10'	15'	60'	120'	24hr
Ph	111±15 (12)	28±18 (12)	66±26* (12)	61±19* (12)	59±17* (12)	60±20 (11)	71±16 (8)	73±16 (7)
S	114±13 (11)	21±14 (11)	25±16 (9)	28±18 (9)	32±20 (9)	52±41 (4)	85±21 (2)	80 (1)

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ACID-BASE BALANCE IN HAEMORRHAGIC SHOCK TREATED BY PHYSOSTIGMINE.
Gordana Žunić, J. Savić, Dj. Prokić, Vesna Selaković. (Spon. J.Savić)
Institute for Medical Research, Military Medical Academy, 11000 Belgrade. Yugoslavia

Physostigmine (Ph) produce prolonged blood pressure rise and survival in haemorrhagic shock, probably due to a centraly mediated increase in the sympathetic discharge. The aim of the present study was to investigate effects of Ph on arterial and/or venous acid-base status in haemorrhagic shock. Haemorrhagic shock is produced in anaesthetized rabbits ($n=12$) using intermittent bleeding of 50% blood volume during 30 minutes. Experimental group (E,n=6) was treated with i.v. bolus (70µg/kg bw) of Ph and control group (C,n=6) with the same volume of saline, immediately after bleeding. Acid-base balance parameters were analysed before, after bleeding (0-min), 15 and 60 minutes after Ph. All animals in E and four in C group survived examined period. Following bleeding arterial pH decreased from 7.409±0.077 to 7.320±0.071 and venous from 7.306±0.109 to 7.185±0.100. After 60 minutes arterial pH was 7.048±0.167 (E) and 7.215±0.047 (C) ($p<0.05$ vs 0-min). Arterial pH, excess base, pCO_2, standard HCO_3^- alterations indicate metabolic origine of acidosis and its respiratory compensation in both groups. Difference between groups are observed in venous blood acid-base balance. The fall in venous pH was larger in E than in C group ($p<0.001$), due to rise in pCO_2 ($p<0.001$). Partly it could be explained in term of vasoconstriction. We concluded that beneficial effects of Ph on blood pressure and survival exist without further acid-base balance disturbances in general circulation.

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METABOLIC ACIDOSIS ACCOMPANYING INFUSION OF VASOACTIVE SUBSTANCES.
K. Ichiyanagi, T. Ishidera, M. Sakai, H. Horikawa (Spon: K. Okada)
Yamagata University School of Medicine, Yamagata, Japan 990-23

Metabolic acidosis accompanies continuous infusion of vasoactive substances. The purpose of this study is to clarify, in the dog, the nature of this metabolic acidosis, which often imposes various problems in the management of shock patients. EXPERIMENT I. METHODS: Twenty mongrel dogs, anesthetized with thiopental infusion, were infused i.v. for two hours with one of four vasoactive substances: epinephrine, norepinephrine, isoproterenol and phenylephrine. The arterial blood was analyzed at intervals for base excess (BE), major metabolic acids, electrolytes and other substances. RESULTS: With each of the four substances metabolic acidosis of a similar magnitude (BE approx. -5 meq/l) developed. Lactate and pyruvate increased with epinephrine and isoproterenol, but not with norepinephrine or phenylephrine. Free fatty acids increased with all but phenylephrine. With epinephrine and isoproterenol changes in the major metabolic acids corresponded well with changes in BE, whereas with norepinephrine and phenylephrine changes in the metabolic acids were so small that they did not match the changes in BE. There were no significant changes in the electrolytes (hence none in the anion gap) with either of the four substances. EXPERIMENT II. In ten other dogs epinephrine infusion was preceded by either an α-blocker (phentolamine) or a β-blocker (propranolol). Development of metabolic acidosis (decrease in BE) and increases in lactate and pyruvate were attenuated by the β-blocker but not by α-blocker. SUMMARY: The nature of metabolic acidosis accompanying infusion of vasoactive substances seems to be determined by their affinity to α- and β-sympathoreceptors.

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318 THE EFFECTS OF STEROID ON HYPOVOLEMIC SHOCK PATIENT ; β RECEPTOR DOWN REGULATION AND IMMUNOLOGICAL SYSTEM . T. Izumi, K. Tanaka, M. Nakamura, K. Suekane. Kinki Univ. School of Medicine, Osaka-Sayama, Osaka 589, Japan

The down regulation of β receptor has been reported on shock patients with long term treatment of cathecholamine. Steroid can reverse this phenomenon in experimental models and clinical researches. However, steroid depresses the immunological system, specially cellular immunity. We studied the hemodynamic changes and the immunological system upon the effects of methylprednisolone(MP;5 mg.kg^{-1}) in hypovolemic shock patients with cathecholamine treatment more than 12 hrs. We administered MP to seven patients in hypovolemic shock, mean blood pressure less than 50 mmHg, urine output less than 0.5 ml/kg/hr with cathecholamine support more than 12 hrs in ICU. After MP treatnent, we measured the hemodynamic changes and immunological system (C_3,C_4,CH_{50}, CD2,CD4, CD8 and CD20) up to 6 hrs. MP increased the mean arterial blood presseure 40 %, urine output 130 %, cardiac index 28 % of pretreatment level without increasing the dose of cathecholamine and fluid replacement by 2 hrs. MP tended to depress the cellular immunity temporarilly , but it could not suppress the immunological system over 6 hrs.We conclude that MP treatment (5 mg.kg^{-1}) can reverse the down regulation of β receptor without the suppress of immunological system in hypovolemic shock patients with long term cathecholamine support.

319 EFFECTS OF DOBUTAMINE ON OXYGEN EXTRACTION IN DOG MODEL OF CARDIAC TAMPONADE.
Zhang Haibo*, Jan Bakker*, Jean-Louis Vincent. Department of Intensive Care, Erasme University Hospital, Free University of Brussels, Route de Lennik 808, B-1070 Brussels,Belgium.

When oxygen delivery (DO2) is reduced below a critical level, oxygen consumption (VO2) declines sharply because tissue extraction is surpassed. This experimental study investigated the changes in VO2 during a reduction in DO2 induced by cardiac tamponade (TAM) in dogs (group C,5 dogs) and the effects on oxygen extraction of dobutamine (DOB) in this model (group D, 6 dogs).
In pentobarbital-anesthetized dogs (weight 22±2kg),femoral and pulmonary artery catheters were inserted for pressure, cardiac output (CO) and blood gas measurements. VO2 was measured from the analysis of expired gases, while pericardial pressure (IPP) was monitored. TAM was induced by continuous intrapericardial infusion of normal saline at an initial rate of 40 ml/h for the first hour and 30 ml/h thereafter. In group D, DOB (10 mcg/kg/min) was started 30 min before intrapericardial infusion.
DOB induced significant increases in CO, DO2 and mixed venous oxygen saturation (SvO2) and decreased in extraction ratio (O2ER) and venous-arterial carbon dioxide difference (dPCO2). During TAMP the fall in CO and DO2 was initially balanced by an increase in O2ER so that VO2 remained unchanged in both group. SvO2 fell from 43±18 to 14±8% in group C and from 61±19 to 24±3% in group D. O2ER increased from 35±10 to 89±5% and from 19±8 to 92±2% in group C and D, respectively. In conclusion, this model is suitable to study the effects of vasoactive agents on the relationship between VO2 and DO2 in low flow states. In this model, DOB can maintain DO2 in the absence of tissue hypoxia but does not increase O2ER when acute circulatory failure has occurred.

S16: Extracorporeal Elimination

320 CASE REPORTS ON PLASMAPHERESIS (P) AS SUPPLEMENTAL TREATMENT REGIMEN IN SEPSIS AND SEPTIC SHOCK. K. Werdan, G. Pilz, S. Kääb, W. Samtleben, H.J. Gurland. Dept. of Medicine I, Grosshadern Munich Univ. Hospital, Munich, Germany.
Based on the rationale of eliminating toxins and mediators in sepsis and septic shock, P was used as a supplemental sepsis treatment regimen.
Patients: 11 patients (3 medical and 8 surgical, mean age: 57.1 years) with sepsis and septic shock (mean Elebute sepsis score: 20.6, mean cardiac index (CI): 5.73, mean systemic vascular resistance (SVR): 397) and severe multiple organ failure (MOF) (mean APACHE II score: 33.0, mortality: 82%) were additively treated with P because of further deterioration despite adequate antibiotic therapy.
Methods: Continuous spontaneous arteriovenous membrane plasma separation with polypropylene hollow fibers was carried out for 2 - 150 h, with an exchange volume of 0.3 - 1.1 l/h and a total of 3 - 119 l. The plasma separated was replaced by albumin and fresh plasma resp.
Results: In 7 out of the 11 patients, neither vascular dysfunction (high CI, low SVR) nor MOF (APACHE II score) showed an improvement within the first 4 days after starting

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P. In 4 patients, however, a prompt rise in SVR (day 0: 335, day 1: 585, day 2: 632) as well as a parallel fall in APACHE II score (day 0: 33.3, day 1: 28.5, day 2: 24.5) could be observed after initiation of this additional treatment. In contrast to the fatal outcome in all of the "non-responders", 2 of the 4 "responder" patients survived. **Conclusions:** 1) In 4 out of 11 patients with sepsis and septic shock deteriorating despite antibiotic treatment, an improvement in peripheral vasodilation and in MOF was remarkable 24 - 48 h after starting P as supplemental therapeutic measure. 2) SVR and APACHE II score may be helpful in identifying these "responders" to treatment. 3) The "response to plasmapheresis" was not superior to the "response to iv immunoglobulin G" that occurred in a comparable patients' group with sepsis and septic shock.

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EFFECT OF HEMOFILTRATION ON HEMODYNAMICS, EXTRAVASCULAR LUNG WATER, AND GAS EXCHANGE IN PATIENTS WITH SEPTIC MULTIPLE ORGAN FAILURE. E. Zadrobilek, V. Evstatieva, R. Függer, H. Andel, and P. Sporn.
Departments of Anesthesia/Intensive Care and Surgery I, University of Vienna, A-1090 Vienna, Austria

Depressed myocardial function, decreased peripheral vasomotor tone, and increased microvascular permeability in septic multiple organ failure (MOF) have been attributed to various inflammatory mediators. Several investigators hypothetized that removal of these substances by hemofiltration would improve these dysfunctions. We studied 14 patients (mean age 48 yr; male/female ratio 10/4; 5 survivors) with MOF secondary to persistent sepsis (4 nosocomial complicating multiple trauma, 10 intra-abdominal) scheduled for renal replacement therapy with predilutional continuous venovenous hemofiltration (CVVH, Amicon Diafilter). Over a 48 h period, the filtration volume averaged 75700 ml/1.73 m^2; mean cumulative fluid balance was +3560 ml/1.73 m^2 (range -5710 to +23090). Blood urine nitrogen and serum creatinine significantly ($p < .001$, Wilcoxon test) decreased from 99 to 66 mg/dl and from 4.6 to 3.0 mg/dl, respectively. There was a significant increase ($p < .05$) of stroke volume index (46 vs 52 ml/m^2), while cardiac index (5.0 vs 5.2 l/min.m^2), mean arterial pressure (76 vs 77 mm Hg), pulmonary artery wedge pressure (9.9 vs 10.4 mm Hg), thermal-dye extravascular lung water (8.7 vs 9.2 ml/kg), alveolar-arterial O_2 tension difference (267 vs 260 mm Hg), pulmonary venous admixture (28 vs 31 percent), O_2 availability index (633 vs 632 ml/min.m^2), and O_2 consumption index (182 vs 181 ml/min.m^2) remained unchanged. CVVH did not lead to a significant reduction in the severity of illness and in inotropic/vasopressor or respiratory support, respectively. In conclusion, pumped (high volume) CVVH provides an effective control of uremia and intravascular volume (with maintenance of a favourable hemodynamic and oxygen transport pattern), but does not improve organ dysfunctions accompanied with persistent sepsis.

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TREATMENT OF SEPSIS WITH EXTRACORPOREAL ELIMINATION OF ENDOTOXIN.
M. Kodama, T. Tani, H. Aoki, K. Hanasawa and T. Yoshioka
Shiga Univ. Med. Sci., First. Dept. Surg., Seta Tsukinowa-cho Otsu Shiga, 520-21 Japan

The main cause of septic shock has been thought bacterial endotoxin. But there has been made sure no evidence about the effectiveness of endotoxin removal from the body. New material, Polymyxin B immobilized to polystyren fiber (PMX-F) has been invented for the removal of endotoxin using extracorporeal circulation. The clinical trial on septic patients using PMX-F column (PMX) have been practiced since 1989. The tentative results of this clinical trial are presented.

Case & Protocol: 15 cases who expressed septic syndrome were done direct hemoperfusion using PMX that was started when septic syndrome was confirmed. Usually one session was practiced for 2 hrs, and its efficacy was evaluated. Blood access was prepared venous to venous with double lumen catheter. Blood flow was 100 ml/min. Anticoagulants were used heparin or nafamostat mesilate.

Results: Cathecolamine administration and ventiratory support were required to maintain BP and bloodgas level in almost all cases. BP and heart rate were improved and blood commpatibilities of PMX were fairy good. The DHP with PMX appeared to be safe even in hypotensive patients. The cardiac index, SVRI, oxygenation and high fever were improved after DHP with PMX. The endotoxin concentration in serum decreased after DHP with PMX ($p<0.05$). Eventually 5 cases out of 15 recovered from septic shock. We will continued this traial to evaluate the efficacy of this new treatment in clinical situations. We believe this will lead to a ture advance in the treatment of septic shock.

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EXTRACORPORAL ADSORPTION OF ENDOTOXIN (ET) IN BLOOD - A FEASIBLE METHOD IN SEPSIS? Staubach, K.-H., Kooistra, A., *Otto, V., *Konstantin, P., Bruch, H.-P.
Dep. of Surgery, *Fresenius AG MTS, St. Wendel, Medical University of Luebeck, Ratzeburger Allee 160, 2400 Luebeck

The incidence of severe sepsis has risen over the last decade. Modern techniques and supportive care in intensive medecine has not decreased mortality but increased the risk of infection. Circulating ET is belived to cause an immune response triggering an inflammatory autoinjury process.

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Extracorporal plasma detoxification by a Polymyxin B (PB) adsorbant (AD) was investigated. The mechanism by which the AD binds LPS has not been defined. Characteristics of our AD are listed in the following table:
As matrix we used a cellulose material with acrylic particles. PB (600 EU/ml) was bound via a DEAE Copolymer spacer to the acrylic particles. The resultant fixed PB stayed firmly attached after washing with different solutions. The blood compatibility was fairly good achieving a more than 90 % platelet recovery with 13 atom spacer chain. A 14 atom spacer chain seemed to be most effective with respect to detoxifying ET achieving a capacity of 150 µgET/ml column material. Increasing the contact time between the blood and the PB ligand by a 13-14 atom spacer chain increased the efficacity of the AD as well as the exposure time and the amount of exposed surface area of the AD. Associated with hemoperfusion, using heparin 5000 U initially and 1000 U/hr thereafter, were coagulation problems. However clearance of ET by specific adsorption during direct hemoperfusion may well be feasible. Our PB-AD appears to be a safe and highly effective device.

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CONTINUOUS ARTERIOVENOUS HEMOFILTRATION (CAVH) THERAPY FOR SEPSIS-INDUCED SHOCK LUNG IN IMMATURE SWINE. J.R. Matson, P.A. Lee, F. K. Straughn, R. W. Pryor, and L.B. Hinshaw. Humana Hospital-Medical City Dallas, Dallas, TX 75230 and Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

The aims of this study were two. First, to determine if CAVH therapy improved morbidity and mortality associated with Staphylococcus aureus (SA)-induced lethal shock lung; second, to determine if toxic mediators are removed by CAVH. In the basic, untreated version of this model, animals died with severe hypoxemia (PaO_2= 45 ± 3) at 23 ± 2 hours following the SA infusion, and at necropsy had severe pulmonary edema and numerous pneumatoceles (bronchoalveolar fistulae). 40 anesthetized swine [20 filtered (F) and 20 nonfiltered (NF)] received an LD_{100} IV infusion of live SA (8 x 10^9 CFU/kg) over one hour. Animals were monitored for 10 hours and observed until death or 7 days. Extracorporeal circulation was begun at the end of the bacterial infusion. In F animals, ultrafiltrate (UF) was sterilely collected (rate=500-1000 ml/hr for 6 hours); fluid was replaced volumetrically (modified Ringer's). F animals were divided into three groups: I-filtration fraction (FF)=8%,n=8; II-FF=25%,n=6; III-FF=39%,n=6. Mean survival time increased significantly with increasing filtration efficiency. Animals in the higher FF group survived up to 138 hours. The mean survival times of the paired NF animals ranged from 30-50 hours. F animals had significantly greater PaO_2 values over time than NF animals. F animals were alert, mobile and feeding normally following the protocol while NF animals were moribund and took water only. Sterile ultrafiltrate collected from the F animals was concentrated and infused into healthy pigs. All UF recipients developed severe hypoxemia and moderate to severe pulmonary edema. UF infusion was lethal in 60% of the recipients. There was no dose response relationship between severity of response to UF infusion and the intensity of filtration of the host, SA-infected animal. Data suggest that CAVH clears harmful mediators produced during sepsis and that CAVH may be beneficial in patients with sepsis or shock lung.

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EXTRACORPOREAL HEMOPERFUSION IN ENDOTOXIN SHOCK. Sándor BENDE, Lóránd BERTÓK[x]
Dept.of Surgery,Semmelweis Hospital, Miskolc, H-3501 Csabai-kapu 9. Hungary,
[x] "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiophygiene, Budapest, H-1775 P.O.Box 101, Hungary

The extracorporeal activated charcoal hemoperfusion was used for elimination of endotoxin in experimental canine endotoxin shock (induced by 1 mg/kg endotoxin i.v.). The endotoxin was labeled with 99mTc. The efficiency of hemoperfusion was tested from the blood samples biologically and isotopically at 15, 30, 60, 90 and 120 min after endotoxin injection. The hemoperfusion can eliminate the majority of circulating endotoxin from the blood within 30 min. On the basis of experimental results the extracorporeal activated charcoal hemoperfusion was used in clinical patients for the elimination of endotoxin from the blood circulation in septico-toxic shock. These clinical trials had sufficient results. The authors recommend the hemoperfusion - as a complementary intervention -in the therapy of septico-toxic (entero-endotoxemic) shock.

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EXTRACORPOREAL ELIMINATION OF PATHOGENIC FACTORS OF SEPTIC MULTIPLE ORGAN FAILURE (SMOF) WITH BLOOD PURIFICATION. H. Shiga*, K. Matsuda*, N. Kitamura*, S. Oda*, Y. Ohtake, T. Sugai* and H. Hirasawa.
Department of Emergency and Critical Care Medicine, Chiba University School of Medicine, Chiba, Japan.

The present study was undertaken to investigate the possibility of the elimination of pathogenic

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factors of SMOF with blood purification. For the extracorporeal elimination we applied continuous hemofiltration (CHF), continuous hemodiafiltration (CHDF) and polymyxin B immobilized fiber (PMX) on SMOF patients. And the efficacy of these blood purifications was studied with the clearance of thromboxan B_2 (TxB_2), lipid peroxide, granulocyte elastase, and C_{3a}. Also studied was the effectiveness of endotoxin removal on cellular oxygen metabolism using DO_2, VO_2 and extraction ratio of oxygen. The clearance of TxB_2, lipid peroxide, granulocyte elastase, and C_{3a} with CHF were 2.53 ml/min, 2.11, 0.35 and 3.76, respectively. On the other hand, those with CHDF were 4.51, 3.11, 0.20 and 3.62, respectively. Those results indicate that each humoral mediator except granulocyte elastase is efficiently eliminated with CHF and CHDF. Endotoxin was removed with PMX. Impaired tissue oxygen metabolism which was provably caused by endotoxin was improved. These results suggest pathogenic factors of SMOF can be eliminated effectively with CHF, CHDF and PMX.

ELIMINATION OF MYOCARDIAL DEPRESSANT SUBSTANCES BY HEMOFLTRATION IN PATIENTS WITH CARDIOGENIC SHOCK. 327

F.Coraim, W.Trubel, R.Ebermann, E.Wolner
Dept. of Anesthesiology an Intensive Care, University of Vienna, Austria

The complete chemical structure of myocardial depressing substances found in patients with low cardiac output syndrome are not completely identified at present. The peptidic structure and a low molecular weight have been assigned, but no further characteristics of chemical structure are known yet. Elimination of such low molecular weight peptides by hemofiltration (HF) seems to play an important role for the patients recovery. Therefore in a comperative study 50 patients suffering from cardiogenic shock after open heart surgery where treated by HF; cardiac function, the hemodynamic behaviour and the clinical outcome were compared to a control group of 47 cardiogenic shock patients without HF treatment. Additionally low molecular weight peptides were isolated from the hemofiltrate of all HF patients. Besides chemical analyses, their negative inotropic effect was demonstrated in a bio-assay using isolated guinea-pig papillary muscle leading to a reduction in contractility in all cases (significant in 86%). In 66% of the HF patients a significant improvement of cardiac function could be observed, compared to a mortality of 76% in the patients without HF. Elimination of low molecular peptides acting as myocardial depressing substances by HF could be demonstrated as well as a significant improvement in clinical outcome after initiation of HF treatment in cardiogenic shock.

EXTRACORPOREAL CIRCULATION: IN VIVO AND IN VITRO ANALYSIS OF COMPLEMENT ACTIVATION BY HEPARIN-COATED SURFACES. 328

M. Kirschfink[1], B. Kovacs[2], K. Mottaghy[2]. [1]Inst. of Immunology Univ. of Heidelberg, Germany and [2]Inst. of Physiology, RWTH Aachen.

Activation of the complement (C) and coagulation/fibrinolysis systems has been shown to contribute complications during extracorporeal circulations (ECC) e.g. with membrane oxygenators. Improvement of the biocompatibility of oxygenator membranes and other ECC surfaces due to positive effects on the coagulation system could be achieved by introducing heparin-coated surfaces. Since heparin is known to have a modulatory effect on the complement system we tested the influence of ECC with heparin-coated surfaces (end-point attachment, CARMEDA AB, Sweden) and without any systemic heparinization versus non-coated surfaces and conventional systemic heparinization on complement. In vivo studies were performed in sheep for up to 5 days using an ECC-system with a capillary membrane oxygenator (Maxima, Medtronic, USA) under standardized conditions. Applying assays for hemolytic function (CH50, APH50) and C3-split products, C activation, occurring predominantly via the alternative pathway, was clearly reduced in sheep connected to oxygenators with heparin-bonded surfaces. In vitro experiments with human serum, circulating in a closed system, showed a marked reduction in C activation by heparin-bonded surfaces, as evaluated by specific ELISAs for C3a, C1rsC1inhibitor (classical pathway) and C3b(Bb)P (alternative pathway). From these results, we conclude that the improvement of biocompatibility of a heparin-coated membrane oxygenator and other ECC components is reflected by the reduction of complement activation.

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S17: Anti Endotoxin Measures and Immunoglobulins

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IMMUNOGENICITY AND ANTIGENICITY OF BACTERIAL LIPOPOLYSACCHARIDES. H. Brade, M. Baumann, L. Brade, Y. Fu, O. Holst, H.-M. Kuhn, M. Lukacova, A. Swierzko. Forschungsinstitut Borstel, Parkallee 22, D-2061 Borstel, FRG.

Bacterial lipopolysaccharides (LPS) are the endotoxins of gram-negative bacteria and represent their major surface antigens. It is desirable to understand the molecular basis of LPS immunoreactivity since it is regarded as a model of how endotoxin interacts with proteins and since antibodies against LPS have the potential to counteract the deleterious effects of endotoxins.

We have combined the tools of structural chemistry and immunochemistry to understand the specificity of antibodies against the lipid A moiety and the core region. The structural similarities of these evolutionary well conserved regions in different bacteria raised already decades ago the question of whether antibodies could be prepared which recognize the similarities of many different LPS, and thus cross-react with a broad variety of gram-negative bacteria.

Despite the fact that antibodies against lipid A cross-react with different lipid A structures which contain the same hydrophilic backbone (phosphorylated glucosamine disaccharide) we were unable to see cross-reaction with bound lipid A, i.e with LPS. Accordingly, we do not expect any protective capacity of anti lipid A antibodies.

According to traditional concepts core antibodies are thought to recognize the core oligosaccharide with the terminal sugar as the immunodominant group. Using chemically defined antigens, we could show that this concept cannot be hold any more. To our present knowledge the majority of antibodies in a polyclonal antiserum after immunization with rough-mutant bacteria recognizes epitopes which comprise both, the core oligosaccharide and parts of the lipid A moiety. Therefore, these antibodies are "LPS-antibodies" in the pure sense of the word.

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FUNCTIONAL AND THERAPEUTIC PROPERTIES OF MONOCLONAL ANTIBODIES (MABS) SPECIFIC FOR THE O POLYSACCHARIDE AND CORE REGIONS OF ESCHERICHIA COLI LIPOPOLYSACCHARIDE (LPS). M. Pollack, W.D. Hoffman, K. Oishi, M. Tao, M.E. Evans, C. Natanson, Department of Medicine, Uniformed Services University of the Health Sciences School of Medicine and Critical Care Medicine Department, NIH, Bethesda, MD, 20814.

We evaluated the binding, complement-fixing, antibacterial, endotoxin-neutralizing, and protective properties of high-affinity murine IgG2a MABS reactive with the polysaccharide O-side chain (O-specific MAB) and core region (core-specific MAB) of E. coli O111:B4 LPS. The O-specific MAB bound extensively to high-molecular weight, O-side chain-containing elements of purified E. coli O111:B4 LPS and to intact homologous bacteria. The core-specific MAB demonstrated limited reactivity with core components of homologous and heterologous smooth LPS and with corresponding whole bacteria, but showed strong reactivity with the rough mutant E. coli J5 strain, corresponding rough mutant LPS, and isolated lipid A. The O-specific MAB deposited C3 on homologous E. coli O111:B4 bacteria, and mediated complement-dependent bacteriolysis and opsonophagocytic killing; the core-specific MAB exhibited none of these activities toward either the E. coli J5 rough mutant or O111:B4 smooth "parent" strain. Neither MAB inhibited LPS-, or lipid A-induced TNF secretion by RAW 264.7 macrophages. Similarly, neither MAB prevented LPS-induced lethality in D-galactosamine-sensitized mice. In contrast, the O-specific MAB prevented LPS-induced mortality in unsensitized mice and protected normal mice against i.p. infections with E. coli O111:B4 bacteria, while the core-specific MAB demonstrated neither in vivo activity. In a canine septic shock model, the O-specific MAB reduced bacterial counts in the blood, circulating endotoxin, and sepsis-related mortality despite its inability to prevent cardiovascular dysfunction. The core-specific MAB, in contrast, had no effect on bacteremia or endotoxemia, but produced sustained improvement in sepsis-associated systemic hypotension and increased survival. The different functional properties of MABS specific for the O-side chain and core regions of bacterial LPS suggest distinct therapeutic roles. While O-specific MABS exhibit striking antibacterial and protective activities against homologous bacteria, core- or lipid A-specific MABS may cross-protect against critical pathophysiologic consequences of sepsis caused by diverse gram-negative bacteria.

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IDENTIFICATION OF WIDELY CROSS-REACTIVE AND CROSS-PROTECTIVE ANTI-LPS CORE MONOCLONAL ANTIBODIES (MAbs). F. Di Padova, R. Barclay*, E. Liehl#.
Preclinical Research, Sandoz Pharma, CH-4002 Basel, Switzerland, *SNBTS, EH3 9HB Edinburgh, Scotland, #Sandoz Forschungsinstitut, A-1235 Vienna, Austria.

In animal models of LPS toxicity, antisera to LPS core have provided conflicting results, and uncertainty on the protective role of anti-LPS core antibodies persists. In the present study we show that high affinity and widely cross-reactive anti-LPS core MAb can be isolated and that these MAbs are able to protect animals from LPS toxicity. The minimal epitope structure recognized by these MAbs is the Rc core of EcJ5. After DOC-PAGE and blotting of smooth (S) and rough (R) LPS, these MAbs react with both the ladder and the core structures, indicating that the recognized epitope is public. These MAbs recognize all clinical isolates of E coli tested so far (more than 80 isolates from patients with bacteremia and infections). At 5-50 ng/ml, they are able to block IL-6 secretion by mouse peritoneal cells induced by S and R-form LPS at concentrations of 50-500 pg/ml. One selected MAb inhibits S and R-form LPS

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induced fever in rabbit (dose 1-3 mg/Kg; time -30 min) and LPS induced mortality in galactosamine sensitized mice (dose 40 mg/Kg; time -2h). Extreme care was taken to avoid LPS contamination of the MAb preparations which were negative in the LAL assay and nonpyrogenic (rabbits). In the fever model this MAb was protective against different preparations of S and R-form LPS from E coli and Salmonella (10-100 ng/Kg) and in the mouse toxicity model against S-form LPS (20-80 ng/Kg). In conclusion we have identified a new class of anti-LPS core MAbs which are widely cross-reactive and cross-protective in relevant in vitro and in vivo endotoxin test systems.

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THE HUMAN MONOCLONAL ANTIBODY HA-1A: STUDIES ON THE EPITOPE LOCATION WITHIN THE ENDOTOXIN MOLECULE AND EPITOPIC EXPOSURE ON THE SURFACE OF VIABLE GRAM-NEGATIVE BACTERIA. W.C.Bogard,* Jr., and S.A.Siegel*, (Spon. H. Redl).

Centocor, Inc., Malvern, Pennsylvania, 19355, USA

HA-1A is a human IgM monoclonal antibody (MAb) raised against the J5 mutant of E. coli. HA-1A has been shown to significantly reduce mortality rate in septic patients [Ziegler, et al., Clin. Res. 38,304A (1990)]. To assess the fine specificity of HA-1A, an enzyme-linked immunosorbant assay (ELISA) system was utilized. HA-1A bound to monophosphoryl lipid A (MPL) prepared from S. minnesota R595 LPS in a dose-dependent manner. The murine MAb (8A1) with a specificity for lipid A and polymyxin B were able to competitively inhibit HA-1A reactivity in the ELISA. HA-1A did not bind to plates which were not coated with MPL nor did any of four negative control human IgM antibodies bind to plates coated with MPL. These results verify that HA-1A reactivity was mediated by specific interactions between HA-1A and an epitope within the lipid A absorbed to the solid phase. Binding could not be demonstrated to smooth LPS in the ELISA format. This observation is consistent with that observed for other anti-lipid A monoclonal antibodies. Only minimal binding to viable gram-negative bacteria could be detected by flow cytometry. However, when the bacteria were first exposed to cell wall active antibiotics, binding of HA-1A was markedly enhanced. These studies show that HA-1A exhibits its broad spectrum activity by interacting with an epitope in the highly conserved lipid A domain of endotoxin. Furthermore, the exposure of this epitope on the cell surface is dramatically increased by membrane disruption with cell wall active antibiotics.

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PHASE I/II CLINICAL TRIAL TO EVALUATE THE PHARMACOKINETICS, SAFETY, AND IMMUNOGENICITY OF MULTIPLE DOSES OF HA-1A (HUMAN MONOCLONAL ANTIBODY) IN HIGH-RISK SURGICAL PATIENTS. M.P. Fink, L.D. Nelson, C.S. Cocanouer, R.C. Straube
Univ. of Massachusetts, Worcester, MA 01655; Vanderbilt University, Nashville, TN; Univ. of Texas, Houston, TX; Centocor, Malvern, PA

HA-1A is a human monoclonal IgM directed against core determinants of lipopolysaccharide (LPS). In a recent multicenter trial, adjunctive therapy with HA-1A was shown to significantly improve survival in patients with gram-negative bacteremia. Based upon these data, the present pilot study was designed to obtain preliminary information regarding pharmacokinetics and efficacy of HA-1A administered as a prophylactic agent to high-risk surgical patients. In this 3-center, prospective, randomized, observer-blinded trial, patients were eligible for entry if they met one (or more) of the following criteria: (a) multiple trauma requiring laparotomy and/or thoracotomy and transfusion of $\geq$ 5 units of packed red blood cells (PRBC) within 24 h; (b) abdominal surgery for bacterial peritonitis (but not meeting criteria for sepsis syndrome); (c) emergency operation for ruptured aortic aneurysm; (d) major ablative procedure requiring transfusion of $\geq$ 5 units of PRBC; severe pancreatitis ($\geq$ 3 Ranson's criteria). Three doses of HA-1A (100 mg each) or placebo were administered on an every 24 h basis. Patients were followed for 28 days or until death. Preliminary results will be presented.

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ANTIBODIES AND GRAM-NEGATIVE INFECTIONS. M.P. Glauser.
Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland

There are at least two rationale for administering intra-venous immunoglobulins (IVIG) to patients at risk for Gram-negative infections: the first is to non-specifically restore IgG levels that are depleted after trauma or surgery. Two recently completed well controlled studies performed in surgical patients have suggested that the prophylactic use of IVIG might decrease the acquisition of new infections, especially the acquisition of pneumonia, and possibly reduce the length of stay in intensive care units. The second rationale for IVIG administration is to supplement the host with specific antibodies directed against various microorganisms. With regard to Gram-negative bacteria, a most common source of severe infections, researchers have attempted to elicit cross-reactive antisera against the core region of endotoxin (lipopolysaccharide, LPS), which is the biologically active part of the molecule, and is highly conserved among Gram-negative organisms. Experimentally and clinically, this approach has been found to be very successful in some studies, while others could not demonstrate that anticore LPS antisera were cross-protective. Antibodies directed against the core region of LPS, particularly against lipid A, are believed to be potentially the most active against the biologic effects of LPS; therefore, two large clinical trials using monoclonal antibodies (MoAbs) have been

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completed in acutely ill patients. The first study used a murine MoAb designated as E5. Retrospective data analysis suggest that the MoAb was possibly protective in patients who were not in shock. The second trial used a MoAb designated as HA-1A. Results show that the MoAb is protective in patients with Gram-negative bacteremia. However, those patients without positive blood culture did not benefit from the administration of HA-1A. Moreover, although believed early during development to be directed against lipid A, the properties of HA-1A are still not fully understood, and its mode of action poorly defined. Since the results of the two studies with monoclonal antibodies are contradictory with regard to the type of patients who might benefit most, further careful studies aimed at defining the clinical indication of these preparations as well as their mode of action should be performed.

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THERAPEUTIC ANTI-LPS IgG OBTAINED BY SCREENING OF BLOOD DONORS.
A. Fomsgaard. Department of Clinical Microbiology, University Hospital of Copenhagen, Denmark.

Therapeutic anti-endotoxin antibodies may be obtained as Mab, by immunization, or by simple screening of blood donors. Although Mab may further prove the concept of immunoprotection by LPS antibodies in shock, optimal effects may be provided by antibodies against more than one LPS epitope. We have characterized anti-LPS IgG obtained by screening. Danish blood donors were screened by ELISAs for naturally occuring IgG antibodies to either a pool of 11 S-form LPSs, to E.coli Ra-LPS, to S.minnesota R60-LPS, or to lipid A. Screening for high titres of one of these types of antibodies also selected sera containing antibodies of the other specificities. Moreover, sera with >40 mg/l of IgG antibodies to the S-LPS pool showed a simultaneous appearence of antibodies specific for S-LPSs other than those screened for. Thus, pooling of high titred sera contributed to a high range of also O-specificities. IgG purified from a pool of 2000 high titred donors neutralized different biological activities of LPS, e.g. the activation of LAL and the induction of TNF. The anti-LPS IgG inhibited lethality induced by LPS in D-galactosamine sensitized mice; protected C3H/TifF mice against otherwise lethal Gram-negative infection; and protected against septic endotoxin shock in burned mice. The protection was IgG dose dependent and caused by specific anti-LPS antibodies. I.v. treatment of patients in septic endotoxin shock with 0.2 mg/kg of the pooled anti-LPS IgG was co-incident with the clearance of endotoxin, the disappearance of serum TNF and improvement in clinical parameters with a decrease in calculated mortality rate. The clinical effect of such anti-LPS IgG are under investigation in a larger double-blinded controlled study.

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BACTERICIDAL/PERMEABILITY- INCREASING PROTEIN (BPI) REDUCES MORTALITY IN EXPERIMENTAL SEPSIS.
Charles J. Fisher, Jr., Steven M. Opal*, Marian N. Marra*, John E. Palardy*, Randy W. Scott*, Center for Critical Care Research, Case Western Reserve University, 2074 Abington Road, Cleveland, Ohio 44106

Gram negative bacteremia (GNB) is associated with the release of lipopolysaccharide (LPS) which triggers release of inflamatory mediators such as tumor necrosis factor (TNF) leading to shock, multiorgan failure and death. BPI is a 55kDa antibiotic polypeptide found in human neutrophil azurophil granules which binds LPS in vitro and inhibits the release of TNF caused by LPS stimulation. Using Sprague-Dawley rats, we tested the in vivo efficacy of BPI against LPS by infusing 500µg/kg of E.coli:0111B4 LPS intravenously (IV) followed 4 hours later with 1mg/kg BPI (IV). Mortality was 8/10 (80%) in untreated control rats compared to 7/21 (33%) of BPI treated rats (x^2 test, P=0.041). Neutropenic pseudomonas bacteremic rats were infused with 10mg/kg of BPI 5 days following pseudomonas challenge compared against a buffer control. None (0%) of the control rats survived compared to 3/5 (60%) of the BPI treated rats. CD1 mice were then challenged with 100mg/kg of LPS 1 hour following either saline or BPI (1mg/kg or 2mg/kg) pretreatment. Mortality of control mice at 24 hours was 75% (3/4) compared to no deaths (0/4) for 1mg/kg BPI and (0/4) for 2mg/kg BPI. High dose BPI toxicity studies revealed no evidence of toxicity when the animals were sacrificed at 7 days. We conclude BPI is a non-toxic naturally occuring protein which binds LPS, inhibits release of TNF and reduces mortality in both LPS and GNB experimental sepsis models. We believe BPI offers a novel immunotherapeutic approach to the management of sepsis

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NOVEL APPROACHES FOR THE INHIBITION OF MEDIATORS OF SEPTIC SHOCK
James W. Larrick, Michael Yen, Michimasa Hirata, Susan C. Wright (Spon: G. Schlag) Genelabs, Redwood City, CA 94063, USA.

Gram negative bacterial infections continue to account for significant mortality and morbidity due the inability of new antibiotics to neutralize the deleterious effects of lipopolysaccharide (LPS) released from the bacterial cell surface. LPS initiates an immunoinflammatory cascade characterized by activation of macrophages and other leukocytes, endothelial cells and parenchymal cells of liver, heart etc. Macrophages are central to this process because they release important mediators of

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tissue damage (i.e TNF, IL1, oxygen and nitrogen free radicals) and initiate coagualation by synthesis of tissue factor. Attempts to inhibit this process by blocking the deleterious effects of endotoxin with for example antibodies have met with limited success. We have purified, sequenced and obtained the cDNA clone of a novel cationic antimicrobial protein originally obtained from leukocytes identified by its capacity to bind to and inhibit various activities of lipopolysaccharide. This protein inhibits LPS induced release of tissue factor and LPS lethality in galactosamine-sensitized mice. A 37 amino acid peptide fragment of this protein (designated RNIP-reactive nitrogen inhibitory peptide) inhibits (IC50 = < 50 nM) LPS and gamma interferon induced nitrogen radical production and TNF release by macrophages. Investigation is underway to determine how RNIP attenuates the activation of macrophages to inflammatory stimuli. The cationic protein, the derived peptide or congeners binding to its receptor may have therapeutic potential for conditions associated with LPS induced tissue injury and activation of mononuclear phagocytes such as shock, sepsis, burns, autoimmune diseases and AIDS.

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HEMODYNAMICS AND SCORING SYSTEMS DURING SUPPLEMENTAL TREATMENT OF SEPSIS WITH IMMUNOGLOBULINS (IG) - RESULTS OF AN OBSERVATIONAL STUDY ON 131 PATIENTS. G. Pilz, O. Bujdoso*, R. Neumann* and K. Werdan. Dept. of Medicine I, Grosshadern Munich Univ. Hospital and *Tropon Vertrieb Cutter, Cologne, Germany.

The effects of supplemental i.v. IG (polyvalent or, in the case of Pseudomonas sepsis, Pseudomonas IG) therapy of sepsis and septic shock on hypercirculation and multiple organ failure (MOF) were investigated in a multicenter study with a total of 131 patients (P) (medical and surgical) by means of systemic vascular resistance (SVR) measurements and of scoring systems (APACHE II, Elebute). In 37 P with a total of 46 septic episodes, invasive hemodynamic monitoring was available ("group 1"); group 2 consisted of the remainder 94 P (95 septic episodes). Pre-treatment sepsis severity was comparable between the 2 groups (Elebute score $\bar{x}$: 18.8 vs 18.9), while pre-treatment MOF was more severe in group 1 (APACHE II score $\bar{x}$: 29.8 vs 20.7; mortality 48% vs 38%).
Results: In about half of the cases ("responders"), a prompt improvement in SVR and APACHE II score was evident from day 0 to day 4 after onset of therapy, thus in close time relationship to the IG administration. This improvement was associated with a better prognosis (mortality 24% vs 58%) and was found in all subgroups: medical vs surgical P; polyvalent vs Pseudomonas IG treatment; severe vs moderate MOF. Among all impaired organ functions, the best improvement occured within the cardiovascular, respiratory and cerebral failure. SVR-increase (>160 dynes*cm^{-5}*sec, >24 hours) and the fall (≥4 on day 4) in APACHE II score were suited to classify "responders" to therapy.

Changes day 0 -> day 4:		non-responders	responders
△ SVR (dynes*cm^{-5}*sec)	(group 1)	-2 (n=24)	+451 (n=22)
△ APACHE II score	(group 1+2)	-0.6 (n=73)	-8.0 (n=67) (1 P not classified)

Conclusion: During supplemental sepsis treatment with IG, about half of the treated patients showed a prompt improvement in hemodynamics and MOF.

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ACUTE RENAL FAILURE, CYTOKINES AND IMMUNOGLOBULIN THERAPY IN SEPTICEMIA
R. Götz, H.G. Kress*, L. Schramm, E. Heidbreder, A. Heidland
Dept. of Nephrology, Medical Clinic and *Dept. of Anesthesiology, University of Wuerzburg

In a prospective randomized study (22 pts. with septicemia of different origin, mean age 57.2±2.7 years) we tried to find out a correlation between the plasma levels of interleukin-2-receptor (IL-2R) and Interleukin 6 (IL-6) and the necessity of dialysis treatment in acute renal failure. Septicemia was defined as leukocytosis/leukopenia, fever, hypotension and beginning respiratory and acute renal failure (ARF). In addition, the randomized immunoglobulin (IgG)-treatment was evaluated. Both groups (IgG vs. Albumin) were similar in age, race and gender distribution, clinical presentation etiology of ARF and pre-existing disease history.
Results: 9 of 22 pts. (68%) died, 15 of 22 pts. (41%) underwent dialysis treatment. After randomization we administered in 10 pts. placebo (albumin), 12 pts. were given IgG (Pentaglobin). 5 of 10 pts. (50%) died in the placebo-group and 4 of 12 pts. (33%) in the immunoglobulin group. Dialysis treatment was necessary in 9 of 10 pts. (90%) in the placebo-group, but only 6 of 12 pts. (50%) in the second group needed dialysis treatment ($p < 0.05$). IL-6 declined significantly in 13 survivors, but remained elevated and increased in 9 non-survivors. Whenever an increase of creatinine/urea was seen, IL-2R raises significantly at the same time. Dialysis treatment hightened plasma-levels of IL-2R, but plasma levels of IL-6 were not influenced. IL-2R decreased significantly after restitution of ARF.
Conclusions: Administration of IgG reduces significantly frequency of dialysis treatment in acute renal failure and septicemia. Only plasma levels of IL-2R correlate to renal function. The further increase after dialysis treatment may be caused by activation of granulocytes or complement. Clinical observation and measuring of IL-2R/IL-6 plasma levels may give you tangible prediction of threatening septicemia and ARF.

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5-S-GLOBULINS AFTER INTRAABDOMINAL TRAUMA. P. Lehmkuhl, S. Jeck-Thole, I. Pichlmayr
Med. Hochschule Hannover, Anästhesiologie IV, 3000 Hannover 51

Intraabdominal infections and trauma are likely to initiate failure of several organ systems. Immunoglobulin therapy is recommended for critically ill patients at an early stage of organ failure. This study had been designed to clarify the profit of 5-S-globulins and of a scoring system to survey the effects of early postoperative immunglobulin therapy. 100 patients received double blind randomised 5-S-globulins (5x150 mg/kg b.w.) or placebo after abdominal surgery and trauma. The Hanover-Intensive-Score (HIS) and the Simplified Acute Physiology Score were used to classify the patients. There were no significant differences in mortality. Nevertheless 6 out of 9 placebo patients and only 1 out of 10 verum patients died from sepsis. Therapeutic effects were documented by HIS. Patients at high risk (HIS 9-12 p.) of the verum group had a significant lower. HIS sum at day 2 and 3 after admission than patients of the placebo group. Patients who positively react to 5-S-globulins showed an average decrease of 5.5 points from day 0 to day 2. On the 7th postoperative day 14 % of the verum group patients required respirator therapy against 53 % of the placebo group patients.

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TOLERANCE TO ENDOTOXIN - A NEW THERAPEUTICAL CONCEPT. K.-H.Staubach, Jonas, S., Kooistra, A., Eilers, J., Schade, U.*, Bruch, H.-P.
Dep. of Surgery, *Forschungsinstitut Borstel, Medical University of Luebeck, Ratzeburger Allee 160, 2400 Luebeck

Endotoxin-Tolerance (ET-T) was initially observed almost 100 years ago by physicians who induced fever by bacterial vaccines for therapeutical therapy. Repeated iv injections of LPS seem to lead to a reduction in the release of the cytokines and be at least partially responsible for ET-T.
In a porcine ET-shock model 6 animals (Gr.1) were given ET-injections in a rising dose of S.a.e. ET (5,10, 30,30 ng daily) prior the ET-challenge of a continuous ET-infusion (0,25 ng/kg/h) and compared to 6 controls (Gr.2) which received only Saline vaccine.
Cardiopulmonary profiles a listed in the following table (mean ± SD):

Group	hour of ET	SVO_2 mmHg	MAP mmHg	CO l/min	HF min-1	paO_2 mmHg
n=6 1	0	53,5(23,9)	84,8(8,1)	2,5(0,2)	66,7(7,5)	122,3(23,2)
n=6 2		63,5(8,5)	75,3(7,2)	2,6(0,4)	59,8(11,2)	129,3(11,4)
n=6 1	2	62,6(5,4)	79,5(14,8)	2,6(0,2)	77,2(7,1)	85,3(28,3)
n=6 2		64,6(4,2)	72,5(7,7)	2,7(0,5)	85,0(2,4)	82,8(11,9)
n=6 1	4	44,4(7,9)	87,8(16,6)	2,3(0,6)	85,2(19,4)	56,0(12,6)
n=6 2		38,3(9,2)	51,5(13,1)	1,8(0,5)	139,8(22,8)	55,8(13,2)
n=6 1	6	42,2(9,5)	74,5(17,9)	2,0(0,4)	94,8(18,5)	57,0(11,7)
n=6 1	8	41,3(10,2)	60,2(28,7)	2,1(0,4)	136,0(45,8)	51,4(12,1)
n=3 1	10	43,8(13,5)	61,0(27,2)	2,0(0,5)	140,3(54,4)	55,0(16,5)
n=2 1	11	54,0(0)	53,0(31,1)	2,2(0)	157,0(73,5)	68,0(0)

The most striking feature of our experiment was the prolongation of survival time from 318 to 618 min. The observed ET-T blocked the development of an acute circulatory failure during endotoxemia. This data indicate that ET-T induced by repeated ET-injections 5 days prior the ET-challenge provides a highly significant protective effect on haemodynamics and survival time. The observation implicates the feasibility of a non-specific immunization.

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PREVENTION OF SEPTIC/ENDOTOXIC SHOCK BY RADIODETOXIFIED ENDOTOXIN (TOLERIN-R) PRETREATMENT. Lóránd BERTÓK
"Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiophygiene, Budapest, H-1775 P.O.Box 101, H u n g a r y

Decreasing of nonspecific resistance is one of the most important facts in the pathogenesis of septic/endotoxic shock. On the other hand the endotoxin is a potent stimulator of immune system but it has many toxic properties. However, the irradiation (60-Co) of endotoxin causes changes its structure and results decrease of toxicity but beneficial properties (e.g. endotoxin tolerance inducing and shock-preventing capacity) are practically unchanged. The irradiated (radiodetoxified) endotoxin (TOLERIN-R) preserved its stimulating effect on the lymphoreticular-immune system and nonspecific resistance. The TOLERIN pretreatment can prevent the majority of animals from the septic/endotoxic shock. The TOLERIN was tested in human beings (5+40 volunteers), too. There experiments demonstrated the innocuity and immunostimulant activity of this preparation. The TOLERIN-R is now under the clinical trials in 9 hospitals on 500 patients. On the basis of the results of the first part of these trials (50 persons) we supposed that the TOLERIN pretreatment can prevent the septic/endotoxic shock and secunder infections in surgerical patients.

S18: Antibiotics and Gut Decontamination

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INTRAABDOMINAL INFECTION AND ANTIBIOTIC SELECTION. N. Shinagawa, J. Yura, M. Muramoto, T. Hayakawa, T. Fukui, K. Mashita, S. Ishikawa, and T. Takaoka
First Dept. of Surg., Nagoya City Univ. Med. Sch., Nagoya, Japan

Peritoneal fluid sampling and its bacteriological examination were performed in 238 patients with perforation peritonitis. Bacterial culture was positive in 100% of patients when the perforations occurred at the colon, whereas it was positive in only 44.4% of duodenal perforations, being negative in many cases when the interval from perforation to surgery was short. Mixed contaminations of both aerobes and anaerobes were usually in the cases of lower digestive tract perforation, but the isolates from duodenal perforations were uniquely aerobes in most cases. Important determinant factors for the prognosis of perforation peritonitis with shock, are early diagnosis and adequet surgical treatment combined with appropriate antibiotic therapy. Once the diagnosis of peritonitis is established, intravenous fluid infusion and antibiotic administration shoule be started immediately. The antibiotic which is chosen before the causative organism is identified, should be a broad spectrum agent with enough efficacy against the respective bowel flora, such as E. coli and the B. fragilis group. At the present, cephems such as cefotiam, cefmetazole, cefoxitin or cefuzonam are suitable as preoperative first choice antibiotics, and once the location of the perforation is clarified by laparotomy, it should be determined whether the chosen drug can cover the bowel flora of that special location.

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ENDOTOXIN LEVELS AND CYTOKINE RELEASE IN GRAM NEGATIVE SEPSIS AFTER STARTING ANTIBIOTIC THERAPY

W. Lingnau, F. Javorsky, M. Duregger, M. Herold, J. Berger, F. Pühringer, N. Mutz
Univ. Innsbruck, Departments of Anesthesiology and Medicine, A-6020 Innsbruck

Gram negative infections and sepsis are associated with many pathophysiologic disorders. Hemodynamic and respiratory effects, fever and hematologic disturbances may lead to multiorgan failure and vasoplegic shock. The use of bactericidal antibiotics under these circumstances often results in a deterioration of cardiopulmonary function. Endotoxins, lipopolyccharides (LPS) of gram negative bacteria have been made responsible for these events. Macrophage activation and cytokine release might promote organ dysfunction.
Patients with clinically and bacteriologically proven gram negative sepsis were included before starting sufficient antibiotic treatment and observed during the following 24 hours. Endotoxin levels were slightly elevated at hour 3 (mean 24.2pg/ml), Interleukin 1 showed a peak at hours 4 to 6 (mean 34.5pg/ml; SEM 2.5), Il6 was high at hours 18 and 19 (mean 1041.4pg/ml; SEM 75.5), whereas TNF did not show changes over the observation period. Il1 and Il6 correlated well with the body temperature. At the beginning of Il6 rise pulmonary shunt volume went up to 40%, peripheral oxygen extraction ratio declined due to low SVR.
Conclusion: After starting an antibiotic therapie in gram negative sepsis endotoxin triggers macrophage activation and may lead to cardiopulmonary deterioration.

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Non-selective gut decontamination (NSGD) in order to avoid bacterial translocation in case of shock

J.Brand, A.Ekkernkamp, A.Helbig, G.Muhr
Chirurgische Universitätsklinik Bergmannsheil, Gilsingstr. 14, 4630 Bochum

Many authors regard bacterial translocation across the gut after a circulatory disturbance as a trigger of multiple organ failure. Can a non-selective gut decontamination (NSGD) reduce the extent of translocation and septic reaction in traumatic shock?

We conducted a propective study in 58 polytraumatized patients with an ISS over 24, in which we compared standard intensive care plus NSGD by orthograde lavage as soon as possible after trauma with standard intensive care without decontamination. Among other items, CRP, elastase, MOF-score, total letality and septic multiple orgen failure were analyzed.

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We found a total letality of 21 percent (6 out of 28) in the control group and 16.6 percent in the group with NSGD.

Septic multiple organ failure happened in 5 out of 28 patients (17.8 percent) in the control group in comparison with 3 out of 30 patients (10 percent) in the group with NSGD.
Orthograde gut lavage for non-selective gut decontamination seems to be a useful prophylaxis against septic multiple organ failure inpolytraumatized patients. The method ist simple and economical.

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SELECTIVE DECONTAMINATION (SD) IN HEMORRHAGIC NECROTIZING PANCREATITIS (HNP)

A.Wechsler-Fördös, C.Armbruster*, G.Edelmann, E.Fischl, M.Kolacny, E.Redl-Wenzl, F.Riezinger**, P.Sporn: Department of Anesthesia, *1st Departement of Surgery, **Pathologic-Bacteriologic Departement, KA Rudolfstiftung, 1030 Vienna, Austria

In HNP, 80% of the fatalities are due to septic complications and infection is the major cause of death beyond the second week. Bacteriologically positive (BP) patients have a three times higher mortality than bacteriogically negative (BN) patients. Endotoxemia also has been documented in HNP and correlates well with multiple organ failure (MOF) and lethal outcome. Up to now, we have studied the effects of SD on prevention of septic complications in 24 patients with HNP grade 3 minimum perioperative TISS Score 50. Method: SD as described by Stoutenbeek, starting with operation.
Results: 9 patients (37,5%) died, 15 (62,5%) survived.

	total	BP	BN
Intraoperative specimen	24	12	12
Survival	15	10	5

10 patients had secondary infection of ncroses, 7 suffered from 11 extraabdominal infections, but there was no pneumonia in 417 days of ventilation. Of the 19 patients surviving more than 2 weeks only 2 died of septic complications.
Discussion: SD was highly effective preventing extraabdominal infections but failed to prevent secondary infection of necroses. But in spite of infection, survival was not lower in the BP group. Possible explanations for these findings controversial to data from literature could be the diminution of the endotoxin-concentration in the gut by the endotoxin-inactivating properties of polymyxin used in SD and also the decrease of endotoxin-release from the gut as conequence of suppression of the gram negative overgrowth by SD. Thus the self sustaining process once endotoxemia has been induced by vasoactive mediators could be interrupted and MOF prevented.

(Spon: G. Schlag)

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CLINICAL EXPERIENCE WITH SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT IN EXTENSIVELY BURNED PATIENTS.

by Mackie D.P., Hertum van W.A.J., Department of Anaesthetics, Rode Kruis Ziekenhuis, Beverwijk, The Netherlands

31 consecutive patients with burns of >30% body surface area (BSA) admitted during a two year period, were treated with a regime of selective decontamination of the digestive tract (SDD), compromising tobramycin (80mg),polymixin E (100mg) and amphoteracin B (500 mg), administered orally four times daily. In addition, cefotaxim (1gm,q.i.d.) was administered parenterally for the first four days. These patients were compared with a similar group of 32 consecutive patients admitted to our unit with burns of >30% BSA in the two years prior to the introduction of SDD. Virtual elimination of gram negative bacteria from the digestive tract was achieved in the SDD group, together with a significant reduction in the incidence of gram-negative wound colonisation. Indices of infectious complications, including mean daily maximum and minimum recorded temperatures, and the incidence of infiltrative changes on chest X-rays were also lower. Clinical resperatory infection and the incidence of septicaemia werereduced. Fewer patients in the SDD group suffered multiple organ failure. There was a marked reduction in the use of systemic antibiotic therapy. These differences were reflected in a reduction in mortality in the SDD-treated patients. No multi-resistent organisms were isolated in the unit during treatment with SDD. This study suggests that SDD may be of real value in the treatment of extensively burned patients.

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PROPHYLACTIC SELECTIVE FLORASUPPRESSION IN THE GASTROINTESTINAL TRACT OF POLYTRAUMATIZED VENTILATED PATIENTS.

W. Lingnau, J. Berger, F. Javorsky, M. Duregger, M. Fille, F. Allerberger, H. Benzer, W. Koller
Univ. Innsbruck, Departments of Anesthesiology and Hygiene, A-6020 Innsbruck

Ventilated severely traumatized patients reach the Intensive Care Unit without infection, their immun system, however, is compromised by the trauma itself and following operations. Barriers of colonisations defence as there are motility and secretions of the gastrointestinal tract and the individual gut flora are impaired. Infections appear in a high percentage and lead via septic syndrom and multiorgan failure to a large amount of late mortality.

Multitrauma patients (ISS mean 34.95, SD 14.3), who are exspected to require mechanical

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ventilation for more than three days, are blindely randomized immediately after entering the emergency room and bacteriological cultures are taken to evaluate the inital flora. Standardized systemic antibiotic prophylaxis with a 4-fluor-quinolone is then started and continued till day four. The local regimen is given until discharge from the ICU.
Resistance patterns of the microorganisms found in these patients did not change over 15 month and compared to not included patients. Koagulase negative staphylococci colonized more often study patients (26% vs. 15%), but did not cause infections. Positive cultures with Candida were found in 7%

PHARMACOKINETICS OF TOBRAMYCIN ON EXTRACORPOREAL MEMBRANE OXYGENATION IN A SHEEP MODEL. J. Möller, J. Gilman*, J. Sussmane*, F.K. Tegtmeyer **350**

*Miami Children's Hospital, Miami, Fl 33155, USA, and Clinic for Pediatrics, Medical University of Luebeck, Kahlhorststraße 31-35, Dw-2400 Luebeck

Extracorporeal Membrane Oxygenation (ECMO) becomes more and more established in severe respiratory failure in neonatal and pediatric patients. Data about pharmacokinetic changes (i.e. half life time-t1/2, volume of distribution-Vd, clearance-cl) are rare and controversial. Severe resp. failure in neonates is often caused by sepsis. Therapy with aminoglycosides is usual and common. We studied changes in tobramycin pharmacokinetics in 11 healthy, infantile sheep (weight: 5-25 kg).
Methods: The day prior to ECMO we infused 2-5 mg/kg tobramycin over 15 min. From a different line we drew blood samples at 10, 20, 30, 45, 60, 90, 120, 180, 240 min post infusion and analyzed tobramycin levels in a commercial kit (Abbott). At day 2 we established ECMO as described by Bartlett. A pre ECMO pre- and post oxygenator blood sample was drawn, ECMO was started, the previous tobramycin dose given, and pre- and post oxygenator samples drawn at the same intervals. Pre- and post membrane as pre- and post ECMO levels were compared using the test for paired samples.
Results: There was no significant difference between pre- and post membrane tobramycin levels. The Vd on ECMO increased compared with day 1 from 0.275 l/kg (SD 0.07) to 0.409 l/kg (SD 0.173) ($p<0.005$). The tobramycin cl did not change significantly, so this was due to an increase in plasma volume.
Conclusion: Opposite to clinical trials we did not find an increased aminoglycoside cl on ECMO. A higher Vd could make higher doses necessary.

S19: Antiproteases and Miscellaneous Drugs

PROTEASE INHIBITORS: THERAPEUTIC APPROACHES IN SEPSIS AND MULTIPLE ORGAN FAILURE **351**
H. Fritz[1], D. Inthorn[2], D. Nast-Kolb[2], M. Siebeck[2], M. Jochum[1] (Sponsor: H. Redl). Dept. Clinical Biochemistry[1] and Surgical Clinics[2] of the University of Munich, D-8000 München, Germany

The release of phagocyte lysosomal proteinases (PMN elastase, macrophage cathepsin B), the activation of proteinases of the blood cascade systems (clotting, fibrinolysis, complement, kallikrein/kinin system), and the consumption of plasma proteinase inhibitors (e.g. antithrombin III) has been shown to correlate well with the clinical situation (multiple organ dysfunctions) of patients suffering from severe trauma and/or septicemia.

In animal experimental approaches proteinase inhibitors against PMN elastase and thrombin have been administered to study the effectiveness of each inhibitor alone or in combination to diminish the inflammatory response in vivo. The results showed a significant reduction of the endotoxin-induced organ dysfunctions in septic pigs by such a proteinase inhibitor therapy.

Moreover, in a pilot study on septic surgical patients, administration of antithrombin III (isolated from human plasma) in high dosages (up to a mean inhibitory activity of 140 % of normal) diminished organ failure and late lethality in those severely ill patients.

THE ROLE OF PROTEINASES IN SEPSIS AND ORGAN FAILURE. H. Neuhof **352**
Justus-Liebig-University Giessen, Department of Internal Medicine, Division of Clinical Pathophysiology and Experimental Medicine, D-6300 Giessen

The role of proteinases in the pathogenesis of sepsis and organ failure has to be

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regarded under two main aspects: specific serin proteinases are main components of the classical cascade systems (complement-, kallikrein-kinin-, coagulation- and fibrinolytic-system), which are activated excessively in sepsis. Some intermediates and end products of these systems are able to influence directly vascular tone and permeability, or to stimulate inflammatory cells for generation and liberation of mediators. Up until now only coagulation and fibrinolysis can be blocked. Inhibitors to prevent or interrupt the activation of the two other systems to the same extent are not yet available.
With regards to cellular, especially vascular injury, lysosomal proteinases seem to play a major role. Main sources of those proteinases, such as elastase and cathepsins, are granulocytes and macrophages. Upon activation, both cell types not only release their proteinases but they also generate reactive oxygen species during their metabolic burst reaction, which inactivate the endogenous α1-proteinase-inhibitor by oxidation. From the vessel side, this dangerous situation occurs when adhering granulocytes release their proteinases in close contact to the vessel wall. Otherwise, a comparable situation develops in the alveoli by activated granulocytes and macrophages. The unlimited lyses of basement membranes and structural elements of the vessel and alveolar walls finally results in the disturbance of vascular and alveolar permeability. Interstitial and alveolar edema formation in the later stages of ARDS and multiple organ failure seems to be caused by a proteolytic process. Since the endogenous proteinase inhibitor in these situations is ineffective, inhibitors which are resistant to inactivation by oxidation are required for prophylaxis and therapy.

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INTERVENTION WITH PROTEASE INHIBITORS IN SEPSIS AND ORGAN FAILURE. EXPERIMENTAL AND CLINICAL STUDIES.

A.O.Aasen, N.Smith-Erichsen, F.Naess and U.Kongsgaard.
Rikshospitalet, The National Hospital, Akershus Central Hospital and Ullevaal Hospital, University of Oslo, 0027 Oslo 1, Norway

The importance of protease inhibitor treatment in endotoxemia was studied both in in vitro experiments and in a pig model. Furthermore the value of antithrombin III (AT-III) substitution was prospectively studied in 83 surgical ICU patients. In the in vitro experiments, doses up to five times the normal plasma content of either AT-III or C1 inhibitor (C1INH) did not prevent activation of the plasma cascade systems by endotoxin. Animals infuced with a combination of three protease inhibitors (AT-III 500IU, C1INH 2000IU and Trasylol 2 mill KIU) prior to the start of the endotoxin infucion revealed increases in functional kallikrein inhibition, antiplasmin and AT-III values to 162, 124 and 114 % of baseline values respectively. In spita of this, plasma kallikrein and plasmin activities increased after endotoxin exposure and only minor differences in hemodynamic parameters were observed compared with endotoxin infuced controls. In the surgical ICU patients receiving AT-III concentrate, plasma AT-III activity was kept constantly at about 100% of normal values. No statistically signifi ant differences in DIC parameters were found between the treatment group and controls. Furthermore no differences in the time of ICU treatment or time of the hospital stay were found.
These studies show that treatment with protease inhibitors alone has only minor or no effect in both experimental endotoxemia and surgical intensive patients.

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ANTI-SHOCK EFFECTS OF SOME PROTEASE INHIBITORS.

T. Oda, M. Miyawaki, K. Kawasaki, T. Sameshima and J. Miyao
Univ. Kagoshima, Kagoshima, Japan

The lysosomes,containing numerous hydrolytic enzymes included proteases, are labilized during circulatory shock. The hydrolyses of intra- and extracellular matrices would play an important role in the pathogenesis of shock. The purpose of this study is to investigate the antishock effects of protease inhibitors .
Methods. Male Wistar rats, anesthetized with 1 g/kg of urethane, were induced hemorrhagic hypotension, a mean arterial blood pressure at 40 mmHg for two hours. The protease inhibitors, aprotinin(10000 u/kg),gabexate mesilate(50 mg/kg) and ulinastatin(50000 u/kg) were given intravenously,and the effects on survival times, alterations of lysosomes and tissue and plasma lysosomal enzyme activities were examined.
Results. The survival times of rats were significantly prolonged in each group compared with untreated control group. The enlargement in size of lysosomes and the elevation of lysosomal enzyme activities within tissue and plasma following hemorrhagic hypotension were significantly inhibited in each treated group.
Conclusion. The protease inhibitors showed antishock properties. And the properties were caused, at least partially, by stabilaization of lysosomes.

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EFFECT OF A NOVEL POTENT BRADYKININ ANTAGONIST IN ENDOTOXIN SHOCK IN RATS. J.C.Cheronis*, J.Blodgett*, S.Eubanks*, K.Nguyen* and E.T.Whalley*. (Spon: M. Seibeck)
Cortech Inc., 6840 N. Broadway, Denver, Colorado, CO 80221, USA.

Bradykinin (BK) has often been implicated in the pathogenesis of septic shock. Early peripheral vascular changes accompanying endotoxic shock or sepsis include a fall in mean arterial blood pressure, events which can be mimicked by BK. In this study we have evaluated the effect of DArg-Arg-Pro-H-Pro-Gly-Phe-Ser-D-Phe-Leu-Arg(CP0088) a compound previously described by Regoli, et. al. (T.I.P.S., 11, 156-161, 1990) and two proprietary analogues of this reference compound, CP0126 and CP0127 on three in vitro tissues and on blood pressure. The effect of CP0127 was then evaluated in a model of endotoxin induced shock in the rat. In vitro studies involved the use of guinea-pig ileum (GPI), rat uterus (RU) and rabbit jugular vein (RJV) where concentration-effect curves to BK were constructed in the absence and the presence of the three antagonists. These studies revealed that CP0126 was equipotent with CP0088. Dose-response curves were then constructed to BK in the anaesthetized rat blood pressure assay in the absence and presence of an infusion of CP0126 or CP0127. Finally, anaesthetized rats were given an i.v. injection of E.Coli endotoxin (LPS 0128:B4) 15mg/kg 5 min after an i.v. infusion of saline or CP0127 50µg/kg/min. CP0127 however was found to be approximately 10 - 100 times more potent than CP0126 on GPI, RU and RJV and also in the rat blood pressure assay. CP0127 was much more difficult to wash off the in vitro tissues and had a longer duration of action in the rat BP compared to CP0126. Injection of endotoxin produced an approximate 50% drop in blood pressure which was maintained for the duration of the experiment (1hr). CP0127 had no effect on the immediate drop in BP seen with endotoxin but produced a total reversal of the subsequent sustained hypotension. On stopping the infusion of CP0127 a slow drop in blood pressure occurred. These results demonstrate that CP0127 is a potent long lasting antagonist of BK in vitro and in vivo and is effective in producing total reversal of the hypotension seen with endotoxin in the rat, an effect not seen with previous BK antagonists. Compounds such as CP0127 may be useful for the treatment of septicaemia in man.

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THE INTERACTION BETWEEN TNF AND ENDOTOXIN IN SHOCK AND PREVENTION OF SHOCK BY COMPLEMENT INHIBITOR (MX-1). K. Matsuda, T. Tani, H. Aoki, T. Yoshioka, M. Kodama.
Department of Surgery Shiga University of Medical Science, Otsu, Japan

In clinical case, patients in septic shock are exposed to both LPS and TNF. Therefore, even if LPS and TNF are not high dose, there is the possibility that the shock is evoked by influencing to each other. In this paper, it was evaluated the synergistic reaction between TNF and LPS in shock and the prevention of shock due to TNF or LPS by complement inhibitor (MX-1).

To test the influence on the toxicity of TNF by the lipopolysaccaride contamination in TNF, TNF and minimal dose of LPS were intravenously injected to S-D rat (200-250g) and mice (ICR-MCH,28-32g). Rats were studied in three groups. These were : 1) only TNF (3.6mg/kg), 2) only LPS (400*3.6ng/kg), and 3) TNF (3.6mg/kg) plus LPS (400*3.6ng/kg). Infusing with TNF plus LPS resulted in a progressive decline in blood pressure with 100% mortality at 4 hours. There were no remarkable changes in the groups of the other. Various concentrations of TNF and LPS were injected into mice. It was observed that all mice were killed by a combined dose (42mcg/Kg of TNF plus 0.5mcg/g of LPS). There were similar findings in another combined dosages.

It was investigated the effect of a complement inhibitor (MX-1) in blocking the adverse changes (loss of body weight, lethality) of TNF or LPS in mice. A complement inhibitor, MX-1, was isolated and purified from the culture of a fungus. MX-1 was effective for inhibiting of body weight loss by TNF (11-83mcg/kg,iv) or LPS (0.04-25mcg/head,ip). The survival rate was improved for all concentrations of TNF. Pathological findings of the lungs of mice which were injected with TNF was improved.

The combination of TNF and a very low dose of LPS increased the mortality of mice and Rat by synergistic interaction between TNF and LPS. In addition, it was ascertained that anaphylatoxin is connected with the activity of TNF and LPS in shock.

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MODULATION OF FIBRINOLYTIC RESPONSE IN PORCINE LPS SHOCK BY PROTEINASE INHIBITORS AND A PAF ANTAGONIST. M. Spannagl, M. Siebeck, H. Hoffmann, H. Fritz, W. Schramm (Spon: H. Redl).
Med. Klinik Innenstadt, Ludwig-Maximilians-Universität, 8000 Munich, F.R.G

Bacterial lipopolysaccharides (LPS) have been shown to release tissue plasminogen activator (tPA) followed by an increase of plasminogen activator inhibitor (PAI) activity in plasma of experimental animals. We studied the effects of pretreatment with various compounds on the LPS-induced increases of tPA and PAI activities in plasma in anesthetized, ventilated pigs versus LPS controls: Hirudin (n 18; n=18), AT III-heparin complex (n=9; n=9), eglin C (n=18; n=18) and the PAF receptor antagonist WEB 2086 (n=10; n=11). All animals demonstrated the typically biphasic time pattern of tPA release (peak at 2 hours) and subsequent increase in PAI activity in plasma. However, hirudin as well as AT III-heparin complex significantly attenuated the decrease of PAI at 2 hours ($p<0.01$) followed by an earlier increase in PAI activity in plasma. In contrast the PAF antagonist WEB 2086 significantly delayed the increase of PAI activity in plasma as compared to the LPS controls. The increase of tPA at 2 hours was significantly higher in the WEB 2086 treated animals ($p<0.05$). Administration of eglin,

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the elastase inhibitor from the medical leech, did not significantly alter the fibrinolytic response to LPS. In summary modulation of tPA and PAI patterns after LPS infusion by inhibition of coagulation enzymes and PAF suggests an important role of thrombin and PAF in the LPS induced activation of fibrinolysis.

358 HIGH-DOSE-APROTININ IN THE PREVENTION OF POSTTRAUMATIC MULTI ORGAN FAILURE

Ch.Waydhas, D.Nast-Kolb, M.Jochum, K.H.Duswald, L.Schweiberer

A prospective pilot study of multiple injured patients was performed to evaluate the effects of high dose aprotinin in the prevention of multi organ failure and the release of mediators and indicators of traumatic shock.

Patients with an ISS $\geq$ 29 points were randomised to receive either a bolus-infusion of 2.5 million KIU aprotinin within the first 2 hrs after the accident, followed by a continous infusion of 1.5 million KIU/h for a period of 24 hrs or the same fluid volume of isotone sodium chloride.

The aprotinin levels in arterial blood of 4 patients rose to 80-360 KIU/ml after the bolus, and peaked at 155 to 500 KIU/ml after several hours. The level was mainly dependent on the initial blood loss. 3 patients with peak levels above 400 KIU/ml showed signs of renal impairment with transient hematuria, decrease of urinary output and increase of serum creatine. These changes were not observed in 6 control patients. Despite the small number of patients the study was stopped at this point.

High aprotinin levels of 200-400 KIU/ml are necessary to inhibit plasma-kallikrein and decrease the release of elastase and other mediators. In studies of open-heart surgery aprotinin has been sucessfully used in high doses.

In multiple injured patients, however, therapy with high dose aprotinin is not feasible since the control of blood levels is not possible due to unpredictable blood loss and lack of bedside tests for aprotinin. Therefore the negative side effects of high dose aprotinin overrule the potential benefits.

359 INHIBITION OF PLASMA KALLIKREIN WITH HIGH DOSE APROTININ IN PORCINE ENDOTOXIN SHOCK.

M. Siebeck, P. Kroworsch, J. Weipert, E. Fink, L. Schweiberer. (Sponsor: H. Redl). University of Munich, D-8000 Munich 2, Germany.

Activation of the contact phase of coagulation has been implicated in the pathogenesis of septic shock. We wanted to determine if aprotinin at plasma concentrations exceeding 400 KIU/ml (≙8.6 µmol/l) provides sufficient inhibition of plasma kallikrein to prevent liberation of kinins from kininogen and arterial hypotension, induced by an infusion of bacterial lipopolysaccharide (LPS) in anesthetized, ventilated 20-kg pigs. LPS was given i.v. in a dose of 5 µg/kg/h for 8 h. Aprotinin, 25 000 000 KIU (≙537 µmol), was given i.v. during 8 h. 10 animals (SA) received LPS and aprotinin, 10 randomized controls (SC) LPS and saline. Kinin-containing kininogen was determined by kinin radioimmunoassay after incubation of plasma samples with trypsin and kininase inhibitors. Results in mean ± SEM. Aprotinin plasma levels were between 468±56 (1 h) and 589±80 (8 h) KIU/ml in SA. Kininogen decreased to 56±7 % of baseline in SA and to 68±5 % in SC at 8 h (p=0.18). Arterial blood pressure at 4 h decreased by 22±5 mmHg in SA and by 38±6 mmHg in SC (p=0.066). Although arterial hypotension was attenuated by aprotinin no reduction of specific kininogen turnover was observed. Therefore, it remains unclear whether the model was inadequate to induce activation of the contact phase of coagulation or whether aprotinin was unable to prevent kinin release from kininogen by plasma kallikrein despite the high plasma levels achieved.

360 ENDOTOXIN SHOCK IN THE PIG:DE NOVO GENERATION OF BRADYKININ B1 RECEPTORS IN VIVO IN THE PULMONARY ARTERY.

E.T.Whalley*, J.Weipert, H.Hoffmann, M.Siebeck, and H.Fritz
Abteilung fur Klinische Chemie und Klinische Biochemie in der Chirurgischen Klinik Innenstadt, Nussbaumst. 20, University of Munich, FRG and *Cortech Inc., 6840 N. Broadway, Denver, CO 80221, USA.

The plasma kallikrein system becomes activated during both experimentally induced endotoxemia and septicemia in man. Bradykinin (BK) activates B1, B2 or B3 receptors. The B1 receptor is not normally present in tissues but can be generated de novo by a variety of inflammatory stimulants and endotoxins. Early vascular changes accompanying endotoxin infusion in pigs include a marked pulmonary hypertension. This study investigates the effect of kinins and other vasoactive agents on pig pulmonary artery (PA) in vitro from control and endotoxin infused pigs. Mini-pigs were anaesthetized and infused with either normal saline or E.Coli (3×10^{10}). Four hours later the animals were killed and the PA removed. Strips of the PA were mounted in tissue baths containing Krebs-Henseleit

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solution at 37°C. After 1hr C-E curves were constructed to BK and the selective B1 receptor agonist des-Arg^9-BK (DABK) and repeated at times 4 and 7hr. Control PA were relatively unresponsive to BK and DABK at t=1hr. Sensitivity and responsiveness to both kinins increased significantly at t=4 and 7hr. The effects of BK and DABK were inhibited by the B1 receptor blocker des-Arg^9-Leu^8-BK. This increase in sensitivity and responsiveness to kinins was not seen in PA incubated with cycloheximide. PA taken from E Coli treated pigs were fully responsive to BK and DABK at t=1hr. Such marked differences in C-E curves to U-46619, PGE_2, 5-HT or ANG II were not seen between control and E Coli treated PA. These results demonstrate that E Coli infusion in pigs can induce de novo bradykinin B1 receptors in the PA which if activated may contribute to the pulmonary hypertension seen in vivo. ETW thanks the Alexander v Humbouldt Stiftung for financial support.

HIRUDIN VERSUS AT III-HEPARIN COMPLEX IN LPS-INDUCED INTRAVASCULAR COAGULATION AND ACUTE LUNG INJURY. M.Weis, H.Hoffmann, M.Siebeck, M.Spannagl, R.Geiger, W.Schramm, H.Fritz (Spon. H.Redl). Ludwig Maximilians Universität, München, FRG.

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Activation of thrombin plays an important role in the pathophysiology of sepsis-induced acute lung injury. In the present study, we examined the effects of r-hirudin *(Plantorgan/Ciba-Geigy)*, a specific inhibitor of thrombin, and of a purified AT III-heparin complex *(Immuno)* on LPS-induced intravascular coagulation (DIC) and acute lung injury in pigs. The pigs were anesthetized, mechanically ventilated, and prepared with Swan-Ganz and extravascular lung water (EVLW) catheters. Three groups of endotoxemic pigs were studied. LPS was given as a continuous i.v. infusion of 10ug/kg/h over a period of 6 hours. Simultaneously with the LPS-infusion the animals received a bolus injection followed by a continuous infusion of r-hirudin (1000 ATU/kg + 500 ATU/kg/h; n=9), AT III-heparin (25 U/kg + 8.3 U/kg/h; n=8), or saline (LPS-Con; n=9). All animal procedures were approved by the Regierung von Oberbayern. The results demonstrated AT III-heparin and r-hirudin significantly prevented the LPS-induced decreases in fibrinogen (AT III-heparin: p=0.0002; r-hirudin: p=0.0003) and increases in soluble fibrin in plasma (AT III-heparin: p=0.0001; r-hirudin: p=0.0049). In addition, r-hirudin treatment attenuated LPS-induced acute lung injury (pulmonary vascular resistance: p=0.0464; EVLW: p=0.0435; peak airway pressure: p=0.0299) and reduced LPS-induced increases in PMN-elastase in plasma (p=0.0433). AT III-heparin neither exhibited an significant effect on LPS-induced lung injury nor on increases in PMN-elastase in plasma. These results indicate both AT III-heparin and hirudin effectively inhibited the proteolytic activity of thrombin generated during LPS-infusion. Furthermore r-hirudin attenuated LPS-induced acute lung injury, whereas AT III-heparin did not. This may be due to the inhibition of direct cellular effects of thrombin e.g. on PMN leukocytes since r-hirudin reduced the release of elastase from activated PMN.

ANTITHROMBIN-III AND HYPERCOAGULATION IN THREE FOALS WITH SEPTICEMIA

B.J. Darien*, and M.A. Williams*† (Spon:J.N. MOORE).
College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331 and College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824†.

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Normal, full-term equine neonates are born with elevated FDP's (10-40 μg/ml) and decreased plasma antithrombin III (AT-III) activity (65% of normal adult levels). AT-III activity and FDP's approach normal levels by 7 days of age. Thus, septicemic equine neonates may be predisposed to thrombotic conditions during the first week of life. Consequently, we evaluated plasma AT-III activity and FDP's in septicemic newborn foals less than 7 days of age before and after treatment with heparin (40 U/kg IV, followed by 40 U/kg SC q12h) and platelet rich equine plasma (3L).

SEPTIC FOAL	PLATELETS (x10^9/L)	PT (sec)	APTT (sec)	FDP (μg/ml)	AT-III (%)	BLOOD ISOLATE	OUTCOME
#1	adequate	13.3	87.6	80 -► 40*	33 -► 61*	*A. equuli*	died
#2	adequat	11.8	61.9	80 -► 40*	46 -► 57*	*E. coli*	lived
#3	adequate	--	--	160 -► 80*	62 -► 62*	*P. aerug.*	lived
Referemce	91 - 325	8.5-11.7	34-47	10-40	60-95		

*Value following treatment with heparin and plasma.

All 3 foals were hypercoagulable at presentation (decreased AT-III activity and increased FDP's), and treatment with heparin and plasma was associated with improvement of the coagulation disorder. The results suggest 1) that foals with gram-negative sepsis warrant evaluation of the hemostasis system and 2) heparin and plasma therapy may help attenuate a hypercoagulable condition.

PLASMA GLUTATHIONE LEVELS IN RATS WITH LIPOPOLYSACCHARIDE-INDUCED LIVER DAMAGE . H. Okabe, K. Irita, M. Yamakawa, J. Yoshitake Dep. Anesth. Crit. Care Med., Kyushu Univ. Sch. Med., Fukuoka 812, Japan

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The plasma levels of reduced glutathione (GSH) were shown to be increased in

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ethanol- or carbon tetrachloride (CCl_4)-poisoned rats. At the same time, the hepatic levels of GSH were decreased. We report here that the plasma levels of GSH increased after lipopolysaccharide (LPS) injection in D-galactosamine (D-gal)-sensitized, starved rats.

Methods: LPS from E. coli, 40 μg and D-gal, 40 mg were injected i.p. in male Wistar rats (200g) starved for 24 hr. Ulinastatin (Uli), a protease inhibitor, at a dose of 50 000 U, was administered i.p. 30 min after the D-gal+LPS injection. The rats were kept starved, and blood samples and the livers were taken 6 hr after the D-gal+LPS injection. **Results**: The plasma levels of GSH were as follows: 16.3±3.0 μM in normal saline-treated rats, 24.8±4.4 μM in (D-gal+LPS)-treated rats, 17.4±3.1 μM in (D-gal+LPS+Uli)-treated rats. The hepatic levels of GSH were 5.4±1.0, 4.6±1.3, and 4.3±1.3 mM, respectively. Uli was also shown to lessen the increase in serum aminotransferase activity, and histological damage.

Conclusion: The increase in the plasma levels of GSH might reflect liver damage induced by LPS as well as by ethanol or CCl_4. Uli was effective in lessening the liver damage.

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MULTITHERAPY ABOLISH ENDOTOXIN-INDUCED LIBERATION OF TNF, BUT NOT OF IL-6.

F. Naess*, A. Waage*, O. Roeise*, J. O. Stadaas*, A. O. Aasen. Dept. of Surgery, Ullevaal Hospital, Oslo, Norway.

The administration of E. coli endotoxin to juvenile pigs in a dose of 0.01 mg/kg over 3 h resulted in a release of TNF occurring as a "burst" with a maximum concentration of 237 ±60 ng/l after 60 min., as determined in a bioassay based on its cytotoxic effect on the mouse fibrosarcoma cell line WEHI 164. TNF concentrations declined to undetectable levels after 180 min. The liberation of IL-6 was bioassayed on an IL-6-dependent mouse hybridoma cell line. IL-6 reached a maximum of 41 ±17 ng/l after 3 h of endotoxemia. One group of animals received a multitherapy regimen aimed at the consiliation of several mediator systems simultaneously, consisting of methylprednisolone, antihistamine, antiserotonin (ketanserin), antiendorphin (naloxone) and three protease inhibitors (C1-inhibitor, antithrombin III and aprotinin) given before endotoxin, and maintenance doses during the study period. This regimen significantly reduced the liberation of TNF to 41 ±33 ng/l, but the liberation of IL-6 was unaffected. Most noteworthy: all hemodynamic parameters were normalized by the multitherapy regimen, whereas the hyperpyrexia induced by endotoxin was not influenced.

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INHIBITION OF THE REPERFUSION PHENOMENA IN CARDIOPULMONARY BYPASS BY DEXAMETHASONE.

W. van Oeveren*, N.J.G. Jansen*, H.M. Oudemans-van Straaten*, C.P. Stoutenbeek, L. Eijsman*, Ch.R.H. Wildevuur*.
University Hospital Groningen and O.L.V.G., Amsterdam, The Netherlands.

In a placebo controlled double blind study on patients undergoing cardiopulmonary bypass (CPB) the effects of dexamethasone (DM) and a placebo were studied on the activation of the plasmatic systems and bloodcells and on the post operative course following CPB.

In the placebo group two patterns of blood activation could be distinguished. From the start of CPB blood-material interaction caused an increase in complement C3a and elastase concentration. After release of the aortic cross-clamp a statistically significant increase was observed in tumor necrosis factor (TNF), leukotriene B4 (LTB4) and tissue plasminogen activator (t-PA) activity ($p<0.01$, $p<0.05$, $p<0.05$ respectively).

DM treatment was not able to inhibit complement activation and elastase release during CPB. However, DM treatment effectively inhibited the increase in TNF, LTB4 and t-PA activity after release of the cross-clamp ($p<0.01$ as compared with the placebo group).

In the postoperative period, the patients in the placebo group suffered from hyperthermia, hypotension and required considerable intravenous fluid administration and cardiotonic treatment. The DM treated patients, however, showed a normothermia ($p<0.01$), had significant higher blood pressures ($p<0.01$) without supportive treatment and consequently stayed shorter in the intensive care unit, which is likely due to the inhibition of leukocyte and t-PA activity after release of the aortic cross-clamp.

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DETRIMENTAL EFFECTS OF HIGH-DOSE METHYLPREDNISOLONE SODIUM SUCCINATE (MPSS) ON HEPATIC AND RENAL FUNCTION IN CLINICAL SEVERE SEPSIS AND SEPTIC SHOCK, GJ Slotman[+], CJ Fisher, Jr.[*], RC Bone[*], TP Clemmer[*], CA Metz, [+]UMDNJ, Camden, NJ, and the Methylprednisolone Severe Sepsis Study Group

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High-dose MPSS may be beneficial in spinal cord injuries but also may increase mortality in acute hepatic failure and systemic sepsis with renal dysfunction. The present study retrospectively evaluated renal and hepatic function in 382 patients with severe sepsis and/or septic shock who received MPSS (30 mg/kg X 4 IV infusions) or placebo in a prospective, randomized, double-blind clinical trial. Hemodynamic parameters and serum levels of creatinine (CR), urea nitrogen (BUN), bilirubin (BILI), mg/dl, and SGOT, u/dl were recorded. Results: Differences in hemodynamic variables, the incidence of shock, and mortality were not statistically significant. Changes in serum indices, compared with admission values (% of patients increased from baseline) 12 and 24 hours after MPSS or placebo were:

	CR		BUN		BILI		SGOT	
	12hrs	24hrs	12hrs	24hrs	12hrs	24hrs	12hrs	24hrs
MPSS	7%	11%	16%*	20%*	24%*	25%*	13%	13%
Placebo	7%	7%	7%	7%	13%	13%	19%	18%

*p ≤0.01, MPSS vs. Placebo

CONCLUSION: High-dose MPSS increases the incidence of renal and hepatic dysfunction in severe sepsis. Hypoperfusion may not mediate this phenomenon. **Adverse effects of pharmacologic MPSS in critically ill man should be considered in planning treatment.**

HISTAMINE RECEPTOR BLOCKERS CIMETIDINE (H2-BLOCKER) AND DIPHENHYDRAMINE (H1-BLOCKER) INHIBIT ENDOTOXIN-INDUCED ELEVATIONS IN PLASMA LEVELS OF CALCITONIN GENE-RELATED PEPTIDE (CGRP). X. Wang*, S.B. Jones, C. Han*, M. Qi*, Z. Zhou* and R.R. Fiscus*. Sanders-Brown Research Center on Aging and Dept. Physiol. & Biophys., Univ. Kentucky College of Medicine, Lexington, KY 40536-0230, Cardiovascular Research Lab., Third Clinical College, Beijing Medical Univ., Beijing, 100083 P.R. China, and Dept. Physiol., Loyola Univ. Medical Center, Maywood, IL 60153. USA

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CGRP, a neuropeptide with extremely potent vasodilator activity, can be released from sensory nerves by certain inflammatory mediators, such as bradykinin, histamine and prostaglandins. Previously, we showed that CGRP is released from vascular nerves into the blood following administration of endotoxin to conscious rats, suggesting that CGRP may play an important role in the hypotension during endotoxin shock. To determine whether histamine is involved in the release of CGRP during endotoxin shock, we tested the effects of two histamine receptor antagonists: cimetidine (40 mg/kg, i.v., H2-blocker) and diphenhydramine (5 mg/kg, i.v., H1-blocker), and the combination of both blockers, given 30 min before injection of endotoxin (lipopolysaccharide B from *S. enteritidis*, 5 mg/kg, i.v.), on CGRP release in conscious rats. Two days prior to endotoxin injection, rats were anesthetized and left carotid artery and right jugular vein were cannulated for measurements of arterial pressure, withdrawal of arterial blood for CGRP determinations and venous injections of endotoxin. Endotoxin, by itself, elevated plasma CGRP levels 7 fold (4.03 ± 0.82 to 30.8 ± 4.4 pg/ml) at 3 hr. Cimetidine and diphenhydramine significantly reduced endotoxin-induced CGRP elevations (19.8 ± 3.3 and 18.7 ± 1.3 pg/ml, respectively). The combination of both H1- and H2-blockers further lowered endotoxin-induced increases in plasma CGRP levels (9.70 ± 3.25 pg/ml, not significantly elevated above control). Hypotension at 30, 60 and 120 min after endotoxin was also partially blocked by cimetidine and diphenhydramine and was almost completed blocked by the combination of both H1- and H2-blockers. Therefore, the data suggest that endogenous histamine, acting at both H1 and H2 receptors, is involved in the release of CGRP as well as the early hypotension during endotoxicosis. The data add further support to our original hypothesis that CGRP, released from vascular nerves (and perhaps other peripheral nerves), serves as a final extracellular mediator of the vasodilation occurring during the pathogenesis of endotoxin shock.

EFFECT OF ANISODAMINE ON ISOLATED RAT LUNG INJURY INDUCED BY OLEIC ACID. De-kun,Song* and Zheng-yao,Lou. Department of Pathophysiology, Hunan Medical University, Changsha, Hunan, 410078 PRC.

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The purpose of this study was to investigate the protective effect of anisodamine (A), a Chinese herb medicine, on the isolated rat lung injury induced by oleic acid (OA). Wistar rats were divided into 3 groups: 1)control (NS); 2) OA group (8ul) and 3) OA+A (addition of A 100mg/L to perfusate 15 minutes prior OA infusion). The results were as follows:

Groups	n	Lung coefficient	MDA (nmol/ml)	SOD (u/ml)	LDH (u/ml)	Lung permeability index
Control	6	25.2±2.5	0.28±0.01	5.89±0.17	14.7±5.2	1.2±0.1
OA	10	8.8±2.2**	0.38±0.02*	2.78±0.23**	71.6±7.4**	2.8±0.2
OA+A	7	10.3±2.3#	0.32±0.01#	3.98±0.34##	33.1±3.9##	1.2±0.1

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* p<0.05, ** p<0.01 vs control group
# p<0.05, ## p<0.01 vs OA group
The results suggested that anisodamine could attenuate the rat isolated lung injury induced by OA.

Plenary Session: Young Investigators Award

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INDUCTION OF HEAT SHOCK PROTEINS IS ASSOCIATED WITH REDUCED MORTALITY IN AN ANIMAL MODEL OF INTRAABDOMINAL SEPSIS. J. Villar, A.S. Slutsky, J.B.M. Mullen (Spon: Heinz Redl). Mount Sinai Hospital, Univ. of Toronto, Toronto, Ontario M5G 1X5, Canada.

Sepsis is the principal cause of multisystem organ failure and death in ICU. We have previously shown that, in animals models, pretreatment with heat induces the synthesis of heat shock proteins (hsp) and attenuates lung damage in a model of acute lung injury. We tested the hypothesis that induction of hsp prior to the onset of sepsis could prevent or reduce organ injury and death in a rat model of intraabdominal sepsis produced by cecal ligation and perforation. Hsp were induced by placing the animals in a neonatal incubator until a rectal temperature of 41-42° C was maintained for 15 min. We studied 30 Sprague-Dawley rats (200-250 gm) randomly divided into two groups: heated (n=14) and unheated (n=16). Mortality rate and pathological changes in lung, heart and liver were evaluated before and 18 h after cecal perforation, 24 h after removal of the caecum, and at 7 days. Heated animals showed a significant increase in hsp70 in the lungs and heart 6-24 h after heat stress. At 18 h after perforation, 25% of the unheated animals died whereas none of heated animals died ($p<0.05$). Heated animals showed a marked decrease in 7-day mortality rate (21%) compared to unheated animals (69%)($p<0.01$). The heated animals showed less histological evidence of lung, liver and heart damage than unheated animals. Although the mechanisms by which hsp exert a protective effect are not well understood, our data raise interesting questions regarding the importance of fever in the protection of the whole organism during bacterial infection.

(Supported by the Medical Research Council, Canada)

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ANTI-TNF MONOCLONAL ANTIBODIES ATTENUATE HEMORRHAGE-INDUCED SUPPRESSION OF SPLENOCYTE PROLIFERATION AND LYMPHOKINE RELEASE. W Ertel, MH Morrison*, A Ayala, IH Chaudry, Department of Surgery, Michigan State University, East Lansing, MI 48824, USA

Although studies have shown that the marked depression of splenocyte (SPL) proliferation and lymphokine synthesis following hemorrhagic shock correlated with high plasma TNF levels, it is not known whether TNF induces the suppression of SPL functions. To study this, C3H/HeN mice were pretreated IP with either a monoclonal TNF-antibody (TNF-Ab) or saline. 20 hr later mice were bled to a mean BP of 35 mmHg, maintained for 1 hr, followed by adequate resuscitation. SPL were prepared at 2 or 24 hr later. After PHA stimulation, SPL proliferation capacity and lymphokine synthesis (IL-2 and IL-6 with bioassays; IFN-γ with ELISA) were determined. TNF plasma levels were measured at 2 and 24 hr after hemorrhage with ELISA. Data shown are 24 hr values (mean ± SEM).

	Proliferation [CPM x 10^3]	IL-2 [U/ml]	IL-6 [U/ml]	IFN-γ [ng/ml]
Sham	273±25	29.7±3.9	1.28±0.09	17.5±1.6
Hemorrhage	148±27*	13.1±1.8*	0.46±0.01*	13.0±1.1*
Hemorrhage+TNF-Ab	237±83	36.3±8.0#	0.94±0.07#	17.1±1.4#

* p<0.05 sham vs hemorrhage; # p<0.05 hemorrhage vs hemorrhage + TNF-Ab; t-test.

Hemorrhage-induced increase of plasma TNF at 2 hr (+215%) and 24 hr (+76%) was attenuated by TNF-Ab. While no decrease in SPL function was observed at 2 hr, a marked suppression of SPL proliferation and lymphokine synthesis was found at 24 hr after hemorrhage. Pretreatment with TNF-Ab significantly increased lymphokine synthesis as well as SPL proliferation. Thus, TNF plays a critical role in initiating and maintaining the depression of SPL functions following hemorrhagic shock.
(Supported by NIH grant R01 GM 37127.)

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EFFECT OF SELECTIVE DIGESTIVE DECONTAMINATION ON HEPATIC MACROPHAGE ACTIVATION AND LEUKOCYTE ADHESION FOLLOWING SMA-SHOCK. R. Hower*, I. Marzi, V. Bähren, and O. Trentz. Department of Trauma Surgery, University of Saarland, D-6650 Homburg/S., FRG.

Ischemia of the gut leads to elevated levels of toxic substances (e. g. endotoxin) during reperfusion period in the

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portal circulation, a mechanism currently discussed to contribute to multiple organ failure (MOF). Removing of gram – negative aerobic flora by selective digestive decontamination (SDD) is an optional therapeutic intervention, however, its effects on macrophage function and leukocyte adhesion in the liver are unknown. In pentobarbital anesthesia (60mg/kg BW i.p.), the superior mesenteric artery of Sprague – Dawley rats was clamped for 60 min. After declamping and a 1 hour reperfusion period, the liver was studied in vivo by means of intravital fluorescence microscopy (Leitz, Wetzlar, 545 nm). Permanent and temporary sublobular leukocyte adhesion to the hepatic sinusoidal wall was determined by i.v. injection of acridine orange (0.5mg). Phagocytic activity of Kupffer cells was assessed following i.v. injection of fluorescence labelled latex beads (0.8 µm diam., 260 * 10^6), allowing determination of the rate of phagocytosis b Kupffer cells. Animals of the SDD – groups received daily antibiotics (polymyxin B 17.1 mg/kg BW, tobramycin 13.7 mg/kg BW, amphotericin 28.5 mg/kg BW) via intragastric instillation during the week prior to the experiments. Hemodynamic parameters, hematocrit and blood gas values were comparable in SMA – shock and SMA – shock/SDD groups. Leukocyte adhesion was increased from 7.1 ± 0.8 % in control group to 23.14 ± 1.9 % in SMA – shock group group, predominantly in periportal and midzonal areas. This effect could be reduced significantly by SDD – treatment to 9.72 ± 0.72 % ($n = 5-6$ per group; Mean ± SEM; $p < 0.05$). The number of pericentrally phagocytosed latex particles increased significantly from 10.54 ± 0.97 in control group to 19.67 ± 2.39 in SMA – shock group and could be reduced by SDD – treatment to 10.65 ± 0.74 ($p < 0.05$). In contrast, the phagocytosis rate in periportal areas did not change significantly following SMA – shock or SDD treatment (19.1 ± 1.95 particles/sublobule in control group, 22.75 ± 2.1 SMA – shock, and 19.83 ± 1.31 SDD – group). In conclusion, selective decontamination of the gut attenuated the increased, shock – induced, leukocyte adhesion in portal and midzonal areas as well as the phagocytic activity in pericentral areas. Removing of potentially pathogenic aerobic gram – negative bacteria could account for this effect, indicating the importance of gut derived toxins for the remote activation of leukocytes and macrophages in the liver.

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BENEFICIAL EFFECT OF H2-AGONISM AND H1-ANTAGONISM IN ENDOTOXIC SHOCK ?
D.Rixen*[1],A.Lechleuthner*[1],S.Saad*[1],A.Buschauer*[3],M.Nagelschmidt*[2], S.Thoma*[2],A.Rink*[2],E.Neugebauer[2]. Surg.Clinic[1],Biochem.and Exper.Div.[2], II.Dept.of Surgery,University of Cologne,D-5000 Cologne-Merheim and Inst. of Pharmacy[3],Freie Univ.Berlin,D-1000 Berlin 33,Germany.

Although histamine (H) is generally considered deleterious in endotoxic shock, several data exist to reevaluate its role: former studies*) showed a possible beneficial effect of H1-antagonism and detrimental effect of H2-antagonism on survival in rat endotoxic shock (REtoxS).Consequently H1- and H2-agonists were studied in REtoxS with the hypothesis of a beneficial effect of H2-agonism and H1-antagonism. Material and methods: 2 randomized studies with H1-and H2-agonists or antagonists on survival-parameters were performed in a standard. REtoxS model (45mg E.coli endotoxin/kg). In both, Methylprednisolone (50mg/kg) and NaCl were used as pos. and neg. controls. Study 1 compared the effects of H1-and H2-agonists (Betahistine:B 0.1mg/kg/h and Impromidine:I 100ug/kg/h) with H1-and H2-antagonists (Astemizole: A and Famotidine:F both 1mg/kg)(20 rats/dose). Study 2 was performed to estimate the dose-response relationship of a new, potent H2-agonist (BU-E 75: 0.01, 0.1, 1.0, 10 and 100ug/kg/h)(20 rats/dose). Results and conclusions: In study 1 a beneficial effect in survival-rate was found for A and I vs NaCl ($p=0.058$). I sign. increased survival-time and -curve ($p=0.01$ and $p<0.05$) compared to F. Study 2 showed a positive dose-response relationship of BU-E 75; survival-rates increased from 30% (0.01ug/kg/h) to 70% (100ug/kg/h);F survival was 35%. These data strongly support the hypothesis of a beneficial effect of H2-agonism and H1-antagonism on survival in REtoxS. The role of H in different phases of shock needs still to be investigated. *) Neugebauer et al., Rev Infect Dis, 5: 585-593. 1987

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DILTIAZEM AND SUPEROXIDE DISMUTASE BENEFICIALLY MODULATE THE HEPATIC ACUTE PHASE RESPONSE DURING SEPTIC SHOCK. S. Rose*, H. Baumann*, O. Trentz and M.M. Sayeed. Loyola University Medical Center, Maywood, IL, RPMI, Buffalo, NY, USA, University of Saarland, Homburg, Germany.

Disturbances in hepatic cellular Ca^{2+} regulation in septic rats can be prevented by treatment with diltiazem (DZ). Preliminary studies have suggested that superoxide dismutase (PEG – SOD) also prevented septic – related cellular Ca^{2+} dysregulation. This study evaluated whether these treatments concomitantly modulate hepatic synthesis of acute phase proteins (APP). Male rats (250g) received intraabdominal implantations of sterile (ST), or bacteria – laden (10^4 CFU B. fragilis + 10^2 CFU E. coli)(SP, fecal pellets (1cc). Some ST and SP rats were treated with DZ (1.2 mg/kg, i.v.) or PEG – SOD (5 kU/kg, i.v.) at 8 hrs after implantation. At 24 hrs post – implantation animals survivability (Surv), body temperature (BT) were recorded, and livers and plasma samples were processed to determine: 1) hepatocyte intracell Ca^{2+} conc. ($[Ca^{2+}]i$ (nM) using fluorescent probe indo – 1, 2) hepatocyte ^{45}Ca uptake ($Ca^{2+}u$) (nmol/mg protein), 3) liver mRNAs for APPs albumin (Alb) and α1 – glycoprotein (AGP) using northern blot hybridization, 4) plasma levels (p) of APPs using rocket immunoelectrophoresis and 5) plasma lactate conc. (Lac)(mM). APP values represent fold – changes from values of livers and plasma of unoperated rats.

	BT°C	$[Ca^{2+}]_i$	$Ca^{2+}{}_u$	AGP_p	AGP_{mRNA}	Alb_p	Alb_{mRNA}	Lac	Surv%
ST	37.7±.1	146± 6	0.14±.01	6.1±0.1	9.2±2.9	0.7±.05	0.7±.15	1.2±.3	100(20/20)
SP	39.8±.2	707±90	0.30±.04	11.3±1.2	25.8±7.7	0.4±.04	0.5±.05	4.9±.7	48(10/21)
SP DZ	38.4±.2	260±29	0.19±.01	7.4±0.1	11.1±2.3	0.7±.02	0.3±.03	2.4±.2	84(16/19)
SP SOD	38.2±.1	199±21	0.19±.01	4.0±0.2	12.7±3.2	0.9±.01	0.8±.05	2.2±.1	87(13/15)

To evaluate direct DZ and PEG – SOD effects, APP transcription and translation were studied, in vitro, using hepatic

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cell lines (H35 and HepG2) stimulated with combined hormones IL-1, IL-6 and dexamethasone. Whereas PEG-SOD did not alter APP synthesis in vitro, DZ caused a 30% inhibition of hepatic APP expression. Overall, DZ and PEG-SOD were beneficial as both decreased plasma lactate, febrile responses and mortality in septic rats. These beneficial effects presumably followed an attenuation of APP response associated with a decrease in cellular Ca^{2+} overload and possibly with a direct effect of DZ on APP gene expression. Whereas DZ could decrease the overload by blocking Ca^{2+} influx through ion specific channels, PEG-SOD could prevent inflammatory signals which augment the channel activity and or membrane Ca^{2+} leaks. (Support: NIH GM32288 & HL31163)

S20: Platelet Activating Factor Antagonists

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REGULATION OF VASCULAR INTEGRITY BY PAF, CYTOKINES AND ADHESION MOLECULES : IMPLICATIONS IN SHOCK AND BURN INJURY.

P. Braquet, D. Hosford, J. M. Mencia-Huerta and M. Paubert-Braquet*.
IHB, 17 avenue Descartes, 92350 Le Plessis Robinson, France and *BIO-INOVA, 48-52 rue de la Gare, 78370 Plaisir, France

In addition to shock, sepsis and thermal injury, several pathologies including asthma, ischemia and graft rejection are characterised by an underlying pathology consisting of endothelial injury, excessive blood cell infiltration and vascular leakage. These alterations contribute to hemodynamic disturbances, edema formation and most notably in shock, organ failure. Interactions between endothelial cells (EC) and circulating cells (CC) are regulated by various mediators which influence the expression of cell surface markers and adhesion molecules. These molecules, particularly leukocyte cell adhesion molecules (Leu-CAM) and VLA 4, appear to play a central role in EC-CC crosstalk. We have shown that PAF primes the synthesis and release of various cytokines such as interleukin 1 and 6 (IL-1, IL-6) and tumour necrosis factor (TNF). In addition PAF enhances the IL-4 induced expression of the low affinity receptor for IgE (FcεII/CD23) on monocytes. We examined the role of PAF in expression of α and common β chains of Leu-CAM's (CD 11a, CD 11b, CD 11c, CD 18), and CD 2/LFA3 (CD 58) on neutrophils and monocytes, by comparing effects of the autacoid with those of IL-4. Both PAF and IL-4 induced a significant increase in CD 11b, CD 11c and CD 18. In contrast, no influence of either PAF or IL-4 on CD 11a expression was observed. Anti IL-4 antibodies inhibited the IL-4-induced expression of CD 11b and CD 11c, while the PAF antagonists BN 52021 and BN 50730 inhibited the PAF-induced appearance of CD 11b, CD 11c and CD 18, a similar effect being observed on CD 2/LFA3 expression. This indication that PAF is implicated in EC-CC adhesion processes is further supported by data showing that antagonists of the mediator can reduce the adhesion of leukocytes to TNF- and IL-1-treated EC by up to 35-40 %. PAF antagonists have already proved therapeutically effective in several animal models of shock and burn, and encouraging preliminary results have been obtained in clinical studies on BN 52021 in burn injured patients.

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ENDOTOXIN INDUCED LOSS OF VASCULAR RESPONSIVENESS; ROLE OF PLATELET ACTIVATING FACTOR.

James R. Parratt, Brian L. Furman and Christine Bouvier*, Department of Physiology and Pharmacology, University of Strathclyde, Royal College, Glasgow, Scotland.

Endotoxemia and sepsis are characterised, at least in the early stages, by impaired vascular responsiveness to both sympathetic nerve stimulation and exogenous vasoconstrictors and vasodilators (Gray et al., Circ Shock,1990, 31, 395-406; Gue et al., Br J Pharmac, 1990. 101. 913-919). This impairment is largely mediated by nitric oxide, or a nitric oxide-like factor, derived from L-arginines (Julou-Schaeffer et al., Am J Physiol, 1990, 259, H1038-1943). However, we do not know whether the generation of NO results from the initial release of other mediators (e.g. Il1, TNF) or from an interaction of endotoxin with specific receptors on the NO-generation cell. Because vascular impairment is also induced by PAF (Gray et al., 1990) we have examined, in anaesthetised and pithed rat, i) whether this impairment is modified by the PAF antagonists WEB 2086 or by an inhibitor of the L-arginine pathway (L-NAME) and ii) whether vascular impairment induced by endotoxin is modified by PAF antagonists such as WEB 2086, in order to determine what part the release of PAF plays in the vascular impairment induced by lipopolysaccharide.

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PAF-ANTAGONIST BN52021 REDUCES HEPATIC LEUKOCYTE ADHESION FOLLOWING INTESTINAL ISCHEMIA. V. Böhren, B. Maier*, R. Hower*, A. Hobmann*, H. Redl§, and I. Marzi. Dept. of Trauma Surgery, Univ. of Saarland, D-6650 Homburg/S., FRG, and §Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria.

Intestinal ischemia followed by reperfusion initiates a variety of systemic inflammatory reactions, e.g. increased

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adhesion of leukocytes to the hepatic endothelium. Free radical scavengers have been shown to reduce intestinal reperfusion injury, however, leukocyte - endothelial adhesion in the liver could not be prevented completely. Therefore, the purpose of this study was, to evaluate if platelet activating factor (PAF) contributes to this phenomenon. Intestinal ischemia/reperfusion injury was induced in anesthetized SPD - rats (250 g) by occlusion of the sup. mes. artery for 60 min, followed by a 60 min reperfusion period (SMA - shock). Following injection of the leukocyte marker acridine orange (0.5 mg), microcirculation and leukocyte adhesion to the sinusoidal endothelial wall was studied by intravital microscopy (Leitz, Wetzlar, FRG; 330x, 545 nm). 4 groups (n = 4 - 6), receiving the indicated treatment during the first 30 min of reperfusion were compared: 1) sham operated control group, 2) shock group (2 ml NaCl 0.9 %), 3) shock/BN52021 group (4 mg/kg of the PAF - inhibitor BN52021 (Dr.Braquet,Inst.Beaufour,Paris), and 4) shock/BN52021 + rhSOD group (40 mg/kg rh - SOD (Grünenthal, Aachen, FRG) additional to BN52021). Macrohemodynamic parameters, hematocrit, and sinusoidal perfusion were comparable in all shock groups. Permanent and temporary (< 20 s) leukocyte adhesion rose after shock induction predominantly in portal and midzonal regions. This effect was reduced by BN52021 treatment alone or combination with rhSOD (see table below).

Adhesion: permanent	portal	midzonal	central	temporary	portal	midzonal	central
Control	3.3 ± 0.7	3.1 ± 1.1	1.3 ± 0.8		4.2 ± 3.1	6.0 ± 1.4	3.5 ± 1.5
Shock	10.7 ± 5.7*	5.5 ± 3.4	2.3 ± 1.8		22.8 ± 6.1*	22.7± 6.6*	6.9 ± 1.8
Shock/BN52021	8.3 ± 2.6	10.3 ± 2.9*	4.5 ± 2.2		7.0 ± 3.6*	0.8 ± 0.7*	4.0 ± 1.7
Shock/BN52021/rhSOD	7.2 ± 2.8	10.5 ± 5.3	7.6 ± 4.1		11.7 ± 8.2	2.7 ± 1.9*	7.3 ± 2.9

Data expressed as percentage, mean ± SD; *, $p<0.05$ shock vs. control; °, $p<0.05$ vs. shock group.

In conclusion, the results indicate that the rise of leukocyte adhesion to liver sinusoids, induced by intestinal ischemia/reperfusion, was largely mediated by PAF. The relationship of PAF and oxygen free radicals requires further evaluation, because no synergistic effect of BN52021 and rhSOD was observed.

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PLATELET ACTIVATING FACTOR (PAF) REVERSES TUMOR NECROSIS FACTOR (TNF) - INDUCED MORTALITY. A PHYSIOLOGICAL ROLE FOR PAF? J. Raymond Fletcher, J. M. Moore,* M. Hubbard*, M. Earnest*, A. DiSimone*, P. Williams*, N. Abumerad.
Vanderbilt University, Nashville, TN 37232 and University of South Alabama, Mobile, AL 36617

PAF primes the cell for release of certain mediators of endotoxemia. Preliminary studies established TNF (150 ng/kg/IV/5 min) caused ~ 90% mortality in 4 hours and that a PAF receptor antagonist decreased the survival time. We hypothesized, therefore, that PAF may be protective in TNF induced mortality. METHODS: Awake male rats with venous and arterial catheters were randomized: Group I - TNF alone (n=30); Groups II and III received PAF receptor antagonist BN52021 (5 mg/kg, IV) + TNF (n=24) or SRI63-875 (20 mg/kg, IV) + TNF (n=28), respectively. Group IV - controls: BN52021 alone (n=3), SRI alone (n=8), vehicles for all agents (n=6 each). Group V - PAF infusions (100-300 ng/kg/min) for 4 hours and TNF. Survival was determined at 2 and 4 hours (see table): *p<0.001 vs TNF alone; ** p<0.01 vs TNF alone at 2 hours.

Survival Percentages

	2 hr	4 hr		2 hr	4 hr
I TNF alone	30	13(4/30)	IV Vehicles/TNF	28	28(5/18)
II BN/TNF	17*	17(4/24)	V PAF(all doses)	90*	90*(26/40)
III SRI/TNF	11*	11(3/28)			

Conclusions: I) TNF produces rapid mortality in rats: II) PAF receptor antagonists enhance TNF mortality (II & III); III) PAF infusions improve (V, IV) survival. PAF may have a physiologic role in TNF induced cellular injury in vivo similar to its primary effect in vitro.

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Aza-alkyl Lysolipids Block TNF-α Secretion by Human Peripheral Blood Monocytes/Macrophages.

Benjamin Bonavida, Anahid Jewett, Hideki Morimoto, Colette Broquet* and Pierre Braquet*, UCLA School of Medicine, University of California at Los Angeles, USA, and *Institut Henri Beaufour, Le Plessis-Robinson, France.

Tumor necrosis factor-alpha (TNF-α) is a 17,000 kda secreted protein produced primarily by monocytes/macrophages. This cytokine has been shown to exert many biological activities and has been implicated in many biological systems including inflammation, immunoregulation, anti-viral and anti-tumor defenses, embryonic development, endotoxic shock, and cachexia. High circulating levels of TNF due to bacterial infections has been associated with the lethal shock syndrome and TNF has been shown to activate latent HIV-1 infection resulting in viral replication and onset of AIDS disease progression. In such disease manifestations, blocking of TNF secretion may result in better prognoses. Our previous studies indicated that PAF antagonists interfere with and down-regulate TNF-α secretion by interferon-gamma (IFN-γ) treated human peripheral blood monocytes (PBM). Further, our recent studies have shown that aza-alkyl-lysolipids (AAL) have cytostatic and cytotoxic effects on tumor cells. The present study investigates the role of several aza-alkyl-lysolipids (AAL) in downregulation of TNF secretion by human PBM derived from normal and HIV seropositive individuals. Normal PBM in the presence or absence of IFN-γ were treated overnight with various concentrations of AAL. The culture supernatants were tested for the presence of TNF by Elisa and by direct cytotoxicity against TNF sensitive target cells using the ^{51}Cr release assay. The response was biphasic. At low centrations, AAL stimulated poorly the production of TNF as detected by Elisa but the TNF biological activity was absent due to the presence of TNF inhibitors. At higher concentrations that were not toxic, AAL inhibited TNF secretion by both untreated and IFN treated PBM. The inhibition of TNF secretion was specific as secretion of another cytokine IL-1β was not significantly inhibited. Previous studies from our laboratory have demonstrated that PBM derived from HIV seropositive individuals and AIDS are de novo activated and constitutively secrete TNF-α in vitro. Treatment of PBM from HIV seropositive individuals with AAL inhibited the spontaneous secretion of TNF-α and also inhibited TNF-α from IFN-γ activated HIV^+ PBM. These results

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demonstrate that AAL downregulate TNF secretion by normal and HIV seropositive derived PBM. This AAL-mediated inhibitory activity may have an important role in regulating TNF-α mediated reactions particularly endotoxic shock, cachexia and induction of HIV from latency. The molecular mechanism of TNF-α downregulation by AAL in PBM and the implications of these findings will be presented.

This work was supported by a gift from Institut Beaufour.

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POST-TREATMENT WITH PAF ANTAGONIST WEB 2086 ATTENUATES PULMONARY DYSFUNCTION IN PORCINE ENDOTOXIN SHOCK. M. Siebeck, J. Kohl, J. Weipert, W. Machleidt, L. Schweiberer. (Sponsor: H. Redl). University of Munich, D-8000 Munich 2, Germany.

The triazolodiazepine WEB 2086, a specific platelet activating factor (PAF) receptor antagonist, has previously been shown to prevent pulmonary hypertension, hypoxia, and bronchoconstriction, when given before an infusion of bacterial lipopolysaccharide (LPS). In the present experiment, we wanted to determine whether WEB 2086 reduced these alterations even when given after onset of LPS shock. In a randomized trial LPS was given i.v. in a dose of 1 µg/kg/h for 8 h to anesthetized, ventilated pigs. 10 animals (SW) received LPS and WEB 2086, 10 mg/kg/h i.v. for 6.5 h, beginning 1.5 h after LPS. 10 septic controls (SC) received LPS and saline. Results at 8 h in % of base line, mean ± SEM. LPS-induced alterations were reduced by WEB 2086: rise in pulmonary artery pressure (SC:188±13; SW:160±10; p=0.05), release of macrophage-derived cathepsin B (SC:436±106; SW:223±32; p=0.041), increase of extravascular lung water (SC:153±26; SW:103±4; p=0.034), increase of airway pressure (SC:181±18; SW:147±8; p=0.055) and rise in alveolar-arterial oxygen gradient (SC:153±22; SW:121±17; n.s.). The PAF antagonist WEB 2086 partially blocked pulmonary dysfunction and enzyme release of inflammatory cells when given during ongoing LPS shock in pigs. PAF possibly mediates some of these changes. PAF antagonists may be useful in the therapy of respiratory failure during septic shock.

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PRE-TREATMENT WITH PAF ANTAGONIST WEB 2086 ATTENUATES PULMONARY DYSFUNCTION IN PORCINE ENDOTOXIN SHOCK. C. Keser, M. Siebeck, J. Weipert, L. Schweiberer. (Sponsor: H. Redl). University of Munich, D-8000 Munich 2, Germany.

We wanted to determine whether the triazolodiazepine WEB 2086, a specific platelet activating factor (PAF) receptor antagonist, reduced pulmonary dysfunction and hypotension during endotoxin shock in anesthetized, ventilated pigs. In a randomized study, bacterial lipopolysaccharide (LPS), 2 µg/kg/h was given i.v. for 6 h. 13 animals received LPS and WEB 2086, 10 mg/kg/h i.v. for 6.5 h, beginning 0.5 h before LPS. 11 septic controls received saline and LPS, 3 non-septic controls saline and WEB 2086, 3 non-septic controls saline only. In 6 animals, we investigated the effect of synthetic PAF in intraarterial bolus doses between 50 and 2000 ng on systemic (AP) and pulmonary (PAP) arterial pressure before and during WEB 2086. LPS produced a PAP rise of 14.5 mmHg; WEB 2086-treated animals had 28% lower PAP than controls (p=0.01). LPS produced systemic hypotension; WEB 2086-treated animals did not differ from controls in AP. LPS-induced leukopenia (p=0.0066), hypoxia (p=0.022), and airway pressure increase (p=0.029) were reduced by WEB 2086. Non-septic controls were stable in all of these parameters. PAF produced an increase in PAP and a biphasic response in AP dose dependently. All PAF dose response curves were right shifted by WEB 2086. PAF was a pulmonary hypertensive agent and contributed to the LPS-induced pulmonary functional alterations whereas its role in systemic hypotension was not confirmed.

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EFFECTS OF ALPRAZOLAM AND STEROID ON ACUTE LUNG INJURY INDUCED BY ENDOTOXIN IN RAT. T. Izumi, K. Tanaka, M. Nakamura, K. Suekane, Y.S.Bakhle*
Kinki Univ. School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589, Japan ,
* Dept. of Pharmacology, Royal College of Surgerns of England

Endotoxin (3.5 $mg.kg^{-1}$) injected intraperitoneally caused leukopenia, lung edema and systemic hypotension in rats. These effects were spontaneously reversed at the 28 hr after the single dose of

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endotoxin. This treatment also slowed the efflux of ^{14}C from [^{14}C]-prostaglandin E_2 (PGE_2) in isolated rat lungs over the same period. These effects of endotoxin were reversed by methylprednisolone (30 $mg.kg^{-1}$), given 30 min after the endotoxin. Alprazolam(10 $mg.kg^{-1}$), PAF antagonist, given 1hr before endotoxin completely prevented the lung edema , systemic hypotension and leukopenia, but did not affect the increased $t_{1/2}$ (time for 50 % of injected ^{14}C to appear in lung effluent) of PGE_2 nor its survival.

[Conclusion] The changes in PGE_2 pharmacokinetics by methylprednisolone could serve as an sensitive index of acute lung injury following sepsis.

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ENDOTOXEMIA IN INTESTINAL SHOCK. EFFECTS OF SOD AND PAF-INHIBITOR.

Eva Haglind, Ragnar Rylander*, Dept. of Surgery, Sahlgrenska sjukhuset and Dept. of Environmental Hygiene, University of Göteborg, S-413 45 Göteborg, Sweden.

The pathophysiology of irreversible shock, following small intestinal ischemia (*ii*) and reperfusion (*rp*), is complex. One important mechanism is the oxygen deficit in the villi, resulting in typical mucosal lesions. Other mechanisms of interest are formation of free radicals and platelet activating factor. Endogenous release of intestinal bacteria/endotoxin has been suggested to be of importance in shock. Using antibiotic pretreatment of shock animals we found no difference in blood pressure or mortality compared to saline pretreated animals. The aim of this study was to determine endotoxin in plasma in animals after *ii* and *rp* and to study the effects of free radical scavenger(SOD/cat) and PAF- inhibitor(BN 52021). **Methods**: 62 Wistar rats were used. Shock was induced in four groups by *ii* (120 cm water for 60 min). One group recieved BN 52021 (BN), 10 mg/kg bw iv 50 min before *ii* , one SOD (5 mg/kg bw)+catalase (20 mg/kg bw) (SOD/cat) 10 min before *rp* , one saline (Sal) in amounts corresponding to SOD/cat and one endotoxin infusion (S-endo) $0.15x10^{-6}$ g as bolus followed by $0.5x10^{-6}$ g (026:B6-LPS, Difco, Detroit, USA). Two groups (no *ii*)served as controls, one without inf.(control), one with endotoxin inf. as above (C-endo) .Blood pressure, plasma volume (PV), intestinal leakage, endotoxin levels (LAL method) were determined. **Results**: All shock groups and C-endo had sign. lower blood pressure compared to control , during and after *ii*. Intestinal luminal leakage was sign. lager in all shock groups compared to control and C-endo. PV was sign larger in control group compared to Sal, SOD/cat and S-endo one h. after *rp* but three h. after *rp* only Sal and S-endo had smaller PV than control. Plasma endotoxin was measured at 0, 60, 120, 240 min in two - six animals in each group. At 0 min the levels were near zero. At 60 min Sal: 0.1±0.1, SOD/cat 1.75±0.7, BN 0.13±0.08, Control $0.05±0.05x10^{-6}$ g. At 120 and 240 min Sal, BN and Control remained stable but SOD/cat increased further to 5.78±0.52 and $2.23±0.41x10^{-6}$ g resp. **Conclusions:** Intestinal ischemia and reperfusion did not result in endotoxemia but this was found after infusion of superoxide dismutase and catalase, as a result of contamination of the enzymes. The importance of this contamination remains to be evaulated.

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PAF IN PATHOGENESIS OF SMAO SHOCK AND THERAPEUTIC EFFECT OF ANISODAMINE

Jing-Yi Su, Qing Chen and Chaoshu Tang
Beijing Medical University, Beijing 100083, PR-China

This work studied the changes of plasma PAF activity and effect of anisodamine(654-2) in SMAO shock in rabbits. SMAO shock was produced by 1hr occlusion and then release of the superior mesenteric artery(SMA). Plasma PAF activity was determined by a revised Henson's method. It showed that plasma PAF activity increased from 3.88±0.82 AU/ml before SMAO to 9.71±1.26 AU/ml($p<0.01$) 2hr after release of SMAO. There was a significant negative correlation between the level of plasma PAF and MABP in the shock animals($r=-0.97$, $p<0.01$). Pretreatment with 654-2(iv infusion of 5mg/kg 654-2 before occlusion of SMA) alleviated hypotension and plasma PAF elevation (from 4.66±0.74 AU/ml increase to 7.06±0.90 AU/ml, compared to the shock group without 654-2 pretreatment, $p<0.05$), also improved survival. Results proved that PAF is an important humoral factor in the pathogenesis of SMAO shock and suggested that 654-2 depressed the synthesis or release of PAF from intestinal ischemia-reperfusion is probably one of the chief mechanisms of its therapeutic action in SMAO shock.

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IMPROVEMENT OF HEPATIC ENERGY METABOLISM FOLLOWING TRANSIENT HEPATIC INFLOW OCCLUSION IN RABBITS BY PRETREATMENT WITH CV6209, A POTENT PLATELET ACTIVATING FACTOR ANTAGONIST.

Y. Takada, H. Higashiyama, S. Iwata, T. Yamaguchi, F. Nishizawa, Y. Shimahara, K. Mori, Y. Yamaoka and K. Ozawa (Spon: H. Hirasawa). Kyoto Univ., Kyoto 606, Japan.

An effect of pretreatment with CV6209, a potent platelet activating factor (PAF) antagonist, on hepatic energy metabolism following transient hepatic inflow occlusion in rabbits was studied. Temporary

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hepatic ischemia was achieved for 30 minutes by cross-clamping of the portal triad without systemic heparinization. Rabbits were divided into 2 groups; those pretreated with CV6209, a potent PAF antagonist, before clamping (CV group, n=5) and untreated group (S group, n=5). Arterial ketone body ratio (AKBR: acetoacetate/3-hydroxybutyrate), reflecting hepatic mitochondrial redox state ($NAD^+/NADH$), and serum lactate level in arterial blood were measured serially after declamping. Also, energy charge of the liver at 60 minutes after declamping were determined. AKBR increased more highly to 1.10±0.05 at 60 minutes after declamping in CV group than to 0.72±0.06 (mean±SEM, $p<0.01$) in S group. Hepatic energy charge at 60 minutes after declamping was significantly higher in CV group than in S group (0.871±0.010 vs. 0.800±0.028, $p<0.05$). Serum lactate level was significantly elevated after declamping in S group as compared with that in CV group. The present study demonstrates the protective effect of pretreatment with CV6209 against the impairment of hepatic energy metabolism following transient hepatic inflow occlusion.

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Contribution of sympathetic nervous system to hypotension induced by platelet-activating factor in anesthetized dogs

Yoshikazu Matsuda and Shozo Koyama
Shinshu Univ. School of Med. Dept. of Physiol. Division 2 Asahi, Matsumoto, Nagano 390, JAPAN

Platelet-activating factor (PAF) is a potent hypotension agent in several species of animals. Intravenous injection of PAF to anesthetized dogs caused marked reductions in systemic blood pressure and cardaic output resulting in circulatory collapse such as endotoxin shock. But, contribution of compensatory response to PAF-induced hypotension remains unclear. In this study, we revaluated contributions of reflex sympathetic responses to PAF-induced hypotension by direct measurement of sympathetic nerve activity on the heart, kidney, liver and adrenal gland. PAF (10 μg/kg) was administrered intravenously in animals with neuraxis intact, all nerve activitieis significantly increased above the control. Activities of the kidney, liver and adrenal gland, through time passes, showed a gradual recovery so that these levels were below the control. However, cardiac nerve activities gradually declined toward the control and were significantly higher above the control. Similar sympathetic nerve responses to PAF were found even in animals with combined denervation of vagal and carotid sinus nerves. These results showed that PAF caused a biphasic response in the sympathetic nerves, indicating that these responses is less dependent on systemic baroreceptor reflex. PAF induced hypotension may be mediated by an interaction between a direct vasodilating effect on peripheral vasculature and sympathoexcitation followed by sympathoinhibiton.

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ACTIVATION OF RAT PLATELETS IN VIVO BY PAF AND SIMULATION OF THIS REACTION IN VITRO.

A. KLEE and D. SEIFFGE
Hoechst AG, Werk Kalle-Albert, 6200 Wiesbaden

Platelet-activating-factor (PAF) fails to induce platelet aggregation in platelet rich plasma of the rat (D. Namm, Thromb. Res. 25: 341, 1982; own results). To evaluate the behaviour of rat platelets in vivo in presence of PAF homologous platelets were labelled using 51Chromium and injected into urethane-anesthetized rats. The distribution of the labelled platelets was continuously monitored using gamma detectors. Count rates and the ratio of two detectors (C1/C2) - one placed above the thorax (C1), the other above the abdomen (C2) - were calculated and displayed by a microcomputer-based system. Intravenous application of PAF induced a rapid sequestration of 51Chromium-labelled platelets in the lung and a concomitant thrombocytopenia. These reactions were dose-dependent and reversible. The pulmonary platelet accumulation was not redeemable by a second application of PAF indicating a desensitization. The function of the platelets, however, was not generally impaired since the platelets were still able to react after injection of ADP. The specific PAF-antagonist WEB 2170 (BOEHRINGER INGELHEIM) inhibited the PAF-induced pulmonary platelet accumulation dose-dependently. In order to imitate this reaction in vitro neutrophils obtained by fractionated density centrifugation were activated by PAF. Addition of the supernatant to washed rat platelets induced a rapid aggregation of the platelets. These results demonstrate that a PAF-specific activation of rat platelets in vivo can occur. This reaction can be mediated by activated neutrophils.

S21: Xanthines—Multifunctional Therapeutic Agents

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NEUTROPHIL CYTOTOXIC ACTIVITY DURING SHOCK AND ITS MODIFICATION BY PENTOXIFYLLINE.
G. W. Schmid-Schönbein and J. Barroso-Aranda.
Univ.of California, San Diego, La Jolla, CA, 92093-0412, USA

Recent results suggest that polymorphonuclear leukocytes (PMN) and monocytes play a central role in cytotoxic tissue reactions in different forms of shock. PMNs can accumulate in the microcirculation, obstruct capillaries, and if activated may mediate cytotoxicity by oxygen free radical formation and release of proteolytic enzymes. During hemorrhagic or endotoxic shock in rats, PMNs accumulate at an early stage in the microcirculation. In hemorrhagic shock the initial trapping is caused by reduction of the perfusion pressure, in endotoxic shock it is caused by activation of circulating PMNs and an increase of their membrane adhesion energy. In short forms of hemorrhagic shock the degree of initial spontaneous PMN activation is an important determinant for survival after shock (Am J. Physiol. 257:H846, 1989). These observations suggest, that an agent which deactivates PMNs and reduces adhesion may be beneficial against tissue injury during shock and improve survival after shock. Direct measurements of PMN adhesion in postcapillary venules shows a reduction by pentoxifylline. Spontaneous superoxide radical formation by PMNs is also impaired by this agent in-vitro and in-vivo. Pentoxifylline treatment of rats before exposure to acute hemorrhagic or endotoxic shock leads to a significantly improved 24 hr survival rate. This is accompanied by a reduced spontaneous activation of circulating PMNs (Biorheology, 27:401-418, 1990). (Supported by USPHS Grant HL10881 and Hoechst-Roussel Pharmaceuticals, Inc.).

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Pentoxifylline in endotoxinaemia in human volunteers

P.Zabel, U.F. Schade, M. Schlaak,
Forschungsinstitut Borstel, D-2061 Borstel. FRG

Pentoxifylline, which is known to have pharmacological effects in animal models of respiratory distress syndrome, multiorgan failure, and shock, was tested in human beings after injection of endotoxin. Of ten healthy volunteers, nine met the inclusion criterion of a rise in body temperature of at least 1,0°C after 100 ng endotoxin (Salmonella abortus equi) as a bolus injection. Serum levels of tumour necrosis factor *alpha* (TNF) and interleukin-6 (IL-6) were both significantly higher than baseline levels 2 h and 3 h after endotoxin injection. 3 weeks later the nine volunteers were again injectet with 100 ng endotoxin and pentoxifylline (500 mg over 4 h) was also infused. There was no rise in TNF levels, though IL-6-levels rose in parallel with body temperature. These data suggest that pentoxifylline blocks the endotoxin induced synthesis of TNF in man and , therefore, could possibly have beneficial effects in clinical endotoxaemia. (Lancet 1989, ii 1474-77)

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ATTENUATION OF ENDOTOXIN-INDUCED ACUTE LUNG INJURY BY PENTOXIFYLLINE AND HWA-138. H.Hoffmann, H.Fritz, M. Weis, F.Aigner, A.Ishizaka, T.A. Raffin, F.W.Schildberg (Spon: H.Redl). Ludwig-Maximilians-Universität, München, FRG; Stanford University, Stanford, CA.

Recently we have demonstrated that pre-treatment with xanthines protected against sepsis-induced lung injury in guinea pigs. In the present study, we examined the effects of post-treatment with pentoxifylline (PTX) or an analogue of pentoxifylline, HWA-138, a xanthine derivative, on endotoxin (LPS)-induced acute lung injury in pigs. Anesthetized, mechanically ventilated pigs, prepared with Swan-Ganz catheters were used. Endotoxin (LPS) was given as a continuous intravenous infusion over a period of 6 hours. 1 h after start of the LPS-infusion the animals received a bolus injection followed by continuous infusion of either PTX, HWA-138, or saline. All animal procedures were approved by the Regierung von Oberbayern. The results demonstrated that post-treatment with PTX or HWA-138 significantly attenuated the LPS-induced increases in the pulmonary vascular resistance, increases in lung wet/dry weight ratios, and increases in the concentration ratio of albumin in bronchoalveolar lavage to that in plasma. In addition, PTX or HWA-138 post-treatment prevented the LPS-induced decreases in lung dynamic compliance and decreases in arterial pO_2. These results indicate that post-treatment with PTX or HWA-138 can attenuate LPS-induced acute lung injury in pigs. Additionally, because PTX and HWA-138 were administered following the septic insult, they may have therapeutic potential.

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COMPARISON OF THE EFFICACY OF DIFFERENT XANTHINE DERIVATES TO REDUCE ENDOTOXIN-INDUCED MORTALITY AND/OR CYTOKINE PRODUCTION: IN VIVO AND IN VITRO STUDIES. S. Bahrami, H. Redl, G. Schlag, G. Leichtfried, M. Ceska* and R.M. Strieter**. Ludwig Boltzmann Inst. Exp. Clin. Traumatol., Vienna, Austria; * Sandoz Research Centre, Vienna, Austria; ** Univ. Michigan, Ann Arbor, USA.

The aim of this study was to compare the potency of three different xanthine derivates (PTX, HWA138 and HWA138-analogous A802715) in order a) to reduce LPS-induced mortality in rats and b) to prevent cytokine production.

Methods: 12 groups of 10 animals each were studied. The rats were injected with LPS (15 mg/kg BW i.p.) and treated either with various doses (40, 80, or 100 mg/kg BW i.p.) of PTX, HWA138 or A802715 or with vehicle as a treatment modality 30 minutes pre-LPS. In vitro studies were undertaken using whole blood (WB) from healthy volunteers incubated for 2/24 hrs following LPS (100 ng/ml) challenge. Each agent was tested at concentrations of 3.5×10^{-6} to 10^{-9} M in 6 replicates. TNF/IL 8 analysis was performed in blood samples at 2/24 hrs post LPS challenge.

Results: The 6-day mortality (75 %) in rats was influenced in a dose-dependent manner. HWA138 reduced the mortality to 22 % as compared to PTX and A802715 (62 %, 55%). In contrast, in vitro studies showed that A802715 is a more potent inhibitor of TNF and/or IL 8 production as compared to PTX or HWA138.

Conclusion: Our data suggest that despite the strong inhibiting effect of xanthines towards cytokine production they might exert additional beneficial effects, which are more pronounced with HWA138, against life-threatening mechanisms during endotoxic shock.

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PREVENTIVE EFFECTS OF XANTHINES ON ACUTE LUNG INJURY IN GUINEA PIGS.

A. Ishizaka, H. Hoffmann* and TA. Raffin**

Department of Medicine, School of Medicine, Keio University, Tokyo, Japan.
* Department of Surgery, Ludwig-Maximilians University Munich, Munich, F.R.G.
**Department of Medicine, Stanford University Medical Center, Stanford, CA, U.S.A.

Although numerous therapeutic agents have been used in the adult respiratory distress syndrome (ARDS) over the past 25 years, none of them has been demonstrated to attenuate the syndrome. Neutrophils (PMN) have been suggested to play a crucial role in ARDS especially in certain etiologies, such as gram-negative sepsis. It has been speculated that a drug, which suppresses PMN activation, might have a preventive effect on PMN related acute lung injury. Xanthines are known to suppress some functions of activated PMN. We evaluated the possible protective effects of xanthines, including pentoxifylline (PTXF), HWA 138 (an analogue of PTXF), and aminophylline (AMPH), in E. coli- and cytokine (tumor necrosis factor; TNF and interleukin 2; IL-2) induced acute lung injury in guinea pigs. In this series of experiments, animals were treated before the insults. The animals were sacrificed and lung tissue sampling and bronchoalveolar lavage (BAL) were performed. Lung damage was assessed by measuring the wet to dry weight ratio (W/D) and the lung tissue and BAL to plasma accumulation of ^{125}I-labeled albumin, referred to as the albumin indices (T-AI and BAL-AI, respectively). ^{51}Cr-labeled red blood cells were injected to correct for blood contamination in the samples. Intravenous administration of E. coli (2×10^9 U/kg), TNF (3.75×10^6 U/kg) or IL-2 (4×10^6 U/kg) caused increases in W/D ($p<0.01$), T-AI and/or BAL-AI ($p<0.01$) indicating that acute lung injury had occurred. Continuous infusion of PTXF attenuated the responses normally seen in acute lung injury following the insults. HWA 138 and AMPH also had a protective effect against E. coli induced lung injury. We conclude that xanthines such as PTXF, HWA 138 and AMPH are effective in preventing acute lung injury due to E. coli, TNF and IL-2 in our guinea pig model.

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XANTHINE-DERIVATES PENTOXIFYLLINE AND HWA 138 ATTENUATES CARDIOCIRCULATORY AND PULMONARY FAILURE SECONDARY TO ENDOTOXIN IN SHEEP.

P.M. Suter, J.-F. Pittet, Ph. Masouyé, D.R. Morel.
Laboratory of Experimental Surgery, University Hospital of Geneva, CH-1211 Genève 4.

Sepsis remains a major cause of multiple organ failure and mortality in ICU patients. By their anti-cachectin activity, certain xanthine-derivates have beneficial short-term effects in endotoxinemia. We examined changes in cardiocirculatory and respiratory function in chronically instrumented sheep, receiving a continuous intravenous E.coli endotoxin infusion (40 ng/kg/min) over 16 hours (1) with and without concomitent infusion of pentoxifylline (PTX) or HWA 138 (2). Both xanthine-derivates similarly prevented endotoxin-induced depression of cardiac output 4 and 8 hours after the beginning of endoxin infusion (4.0 and 4.2 versus 2.5 and 1.6 l/min), increase in pulmonary vascular resistance (330 and 310 versus 920 and 1250 dynes.sec/cm^{-5}). In contrast, HWA 138 increased total oxygen consumption more markedly than PTX (550 and 650 versus 480 and 540 ml/min) at 4 and 8 hours. The increase in plasma lactate was not prevented by the agents tested, but markedly delayed.

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These results suggest that PTX and HWA 138 attenuate cardiocirculatory depression by endotoxin, allow to maintain higher oxygen delivery and consumption, and decrease mortality in chronic endotoxinemia in sheeps.

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EFFECT OF XANTHINE DERIVATIVES ON CAMP, TNF AND CATHEPSIN B PLASMA LEVELS IN PORCINE ENDOTOXIN SHOCK. A. Birg, M. Spannagl, S. Endres, H.J. Fülle, W. Machleidt, H. Hoffmann (Spon: H. Redl).
Med. Klinik Innenstadt, Ludwig-Maximilians-Universität, 8000 München, FRG

Xanthine derivatives have been shown to suppress the release of TNF from leukocytes in vitro and in vivo. Therefore a protective effect of these agents has been supposed in endotoxin shock. We investigated the effect of early posttreatment with pentoxifylline (PTX) or HWA-138, an analogue of PTX, in miniature pigs during a continuous i.v.-infusion of LPS fom *S. abortus equi* (2 µg/kg/h) over a period of 6 hours. One hour after the beginning of the LPS-infusion the animals received a bolus injection of PTX, HWA-138 respectively (6 mg/kg; 3 mg/kg) followed by a continuous infusion (PTX 3 mg/kg/h, n=9; HWA-138 1.5 mg/kg/h, n=9). LPS control group animals (n=9) received only saline. We determined the plasma levels of cAMP and TNF by using specific immunoassays; cathepsin B activity in plasma was measured by a chromogenic substrate assay. Plasma cAMP levels increased steadily during the experiment both in the PTX and HWA-138 treated groups, whereas LPS controls exhibited a moderate increase at the end of the experiment. Similarly cathepsin B activity in plasma increased steadily during the experiment. However this increase was significantly attenuated in both treated groups ($p<0.05$). TNF plasma levels peaked between 30 and 50 minutes after LPS and then decreased slightly until the end of the experiment. No statistically significant difference was detected. The results indicate a rapid release of TNF after LPS-infusion, that was not affected by posttreatment with PTX or HWA-138. However, both PTX and HWA-138 reduced LPS-induced increases in cathepsin B activity in plasma, suggesting a preventive effect on macrophage activation in vivo.

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INDUCED MACROMOLECULAR PERMEABILITY IN THE RAT MESENTERY AND THEIR PHARMACOLOGICAL INHIBITION. **D. Seiffge and V. Laux.**
HOECHST AG, Werk Kalle-Albert, Inst. of Pharmacology, FRG and Univ. of Mainz, Inst. of Zoology, FRG.

Many disease states, particularly those involving acute and extensive injury, hemorhage or sepsis are characterized by a gradual increase in vascular permeability, primarily in the microcirculation. In the last years there had been many attempts to quantify macromolecular permeability in various tissues and organs. Most of them allow only semiquantitative measurements (e.g. counting of leakage sites in the hamster cheek pouch) or are final point assessments. With development of video-image analogous and digital processing a continuous measurement of the extravasation of fluorescence-tagged macromolecules is possible. We have developed a method using FITC-tagged autologous rat serum albumin to study induced vascular permeability in the microcirculation. Using analogous video-image analysis system we have studied the effect of various mediators on microvascular permeability in the mesentery. We found that histamine, platelet activation factor (PAF) and lipopolysaccharide (LPS) are leading to an increase in permeability in postcapillary venules, not in arterioles and capillaries. While histamine exerts a direct effect on the endothelial cell, the effect of PAF and LPS seems to be mediated by leukocytes. Increased permeability induced by histamine could be attenuated by the H_1-antagonist pheniramine, but not by the H_2-antagonist cimetidine. Also, PAF-induced permeability could be inhibited by a specific PAF-antagonist, or LPS-induced venulare leakage was ameliorated by the xanthine derivatives pentoxifylline or HWA 138 dose-dependently.

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COMPARISON OF DIFFERENT XANTHINE DERIVATES ON MONOCYTE AND GRANULOCYTE FUNCTION IN VITRO. **R. Kneidinger, E. Paul, H. Redl and Schlag G. Ludwig Boltzmann Institute Exp. Clin. Traumatol., Vienna, Austria**

It was found that pentoxyfylline (PTX) (a methylxanthine) exerts positive

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therapeutic effects in shock and sepsis, which is probably due to PTX action on white blood cells. Recently, new xanthine derivates have been synthesized (eg. HWA138, HWA2715). We became interested to compare these substances with pentoxyfylline in terms of inhibition of production of cytokines and release of PMN elastase dependent on the employed endotoxin concentration at a consistent xanthine concentration of 100 µg/ml. Heparinized blood was incubated with endotoxin at 37° C for 4^h or 24^h (11 different volunteers).

Results: % of baseline (10 ng LPS/ml without inhibitor = 100 %

	TNF (4^h)	IL-1 (24^h)	Elastase (4^h)
PTX	23.15 ± 13.55	84.77 ± 1.59	54.21 ± 17.82
HWA138	27.70 ± 19.01	73.84 ± 9.81	55.86 ± 6.84
HWA2715	19.03 ± 5.42	93.49 ± 13.36	61.55 ± 20.80

Conclusions: TNF release was most efficiently blocked, followed by elastase and IL-1. IL-1 and elastase release were blocked to a similar extent at both LPS concentrations, whereas TNF-release was more effectively inhibited at the low LPS-dose. Among the tested substances HWA2715 was the most potent TNF inhibitor (less effective with IL-1 and elastase), while HWA138 tended to block IL-1 and elastase release more efficiently. The different blood donors had a highly variant individual sensitivity to xanthine effects. Therefore no clear conclusion regarding the most effective blocker of TNF, IL-1 and elastase release can be drawn from this in vitro experiment.

397 XANTHINE DERIVATIVES SUPPRESS LPS-INDUCED IN VITRO- PRODUCTION OF TNF α IN HUMAN MONONUCLEAR CELLS VIA ELEVATION OF cAMP.

S. Endres, B. Sinha, D. Stoll, H.-J. Fülle, and M. M. Schönharting[1]
(Spon: H. Redl)
Medizinische Klinik, Klinikum Innenstadt, Universität München, and[1]Hoechst AG, Werk Albert, Department of Clinical Research, Wiesbaden, FRG

We compared the in vitro-effect of the following xanthine derivatives on the LPS-induced TNFα production in freshly isolated human mononuclear cells: caffeine, theophylline, pentoxyfylline, penthydroxyfylline, and the related agents A802715, HWA 138 and HWA 448. All substances suppressed TNFα production in a dose-dependend manner at concentrations ranging from 8 to 1000 µM. Inhibition to 50 % of control was reached, in order of potency, for A802715 at 33 µM, for penthydroxifylline at 105 µM, for HWA 138 at 106 µM, for pentoxifylline at 120 µM, for HWA 448 at 280 µM, for theophylline at 345 µM, and for caffeine at 520 µM (means of three to seven individuals, with high interindividual reproducibility).
Suppression of TNFα production was paralleled by a dose-dependent elevation of total (i. e. intra- and extracellular) cAMP levels. We conclude that this suppressive effect of the xanthines, which act as inhibitors of cAMP-degrading phosphodiesterases, is mediated by accumulation of cAMP. The beneficial effect of xanthine derivatives in animal models of septic shock may be mediated, in part, by suppression of TNFα synthesis.

398 EFFECTS OF PENTOXIFYLLINE ON OXYGEN TRANSPORT IN HUMAN SEPTIC STATE.

H. Steltzer, N. Mayer, E. Sposta, P. Germann, J. Winternitz, C. Oismüller and A.F. Hammerle (Spon: G . Schlag).
Department of Anesthesia and General Intensive Care, Sepsis Research Group - University of Vienna, A-1090 Vienna

Pentoxifylline (PTF) has been found to have a variety of pharmacologic effects which could be of benefit in sepsis. Previous work has shown that PTF increases red blood cell deformability (1) rising the speculation that an improvement in blood flow and oxygen delivery in previous constricted microcirculatory areas characteristic in sepsis could result from its application. The goal of the present study was to investigate the effects of PTF on oxygen transport with special reference to the improvement of tissue oxygenation. **METHODS:** We investigated six patients after fulfillment of established sepsis criteria. Measurements were made at 5 intervalls: control, and after 30, 90 , 150 and 210 minutes. PTF (5mg/kg) was given intravenously over a period of 3 hrs. Direct measurements of cardiac output, arterial and mixed venous oxygen content, saturation and hemoglobin were made before (control) during (30,90,150min) and after (210min) infusion of PTF. Oxygen delivery (DO2) and -consumption (VO2) were calculated according to standard formulas. statistical analysis was performed using analysis of variance with Tukey´s method for multiple comparisons. **RESULTS:** By focusing on oxygen transport parameters and oxygen consumption no statistical significant different pattern was found. However, oxygen consumption and transport increased slightly during PTF infusion (VO2:158±23, control and 194±35ml/min/m^2 during PTF, n.s.; DO2: 611±148 and 749±108ml/min/m^2 , n.s.). **CONCLUSION:** PTF has been widely tested for its action in improving blood flow and normal cellular function depends upon a supply of oxygen adequate to meet metabolic needs. However, in our group of patients DO2 as a product of arterial oxygen content and blood flow did not improve and VO2 did not significantly increase during and after infusion of PTF. These findings are in contrast to conclusions derived from investigations in canine shock models (1). Thus, in septic states the gross microcirculatory dearrangements seem to weaken the beneficial responses of regional blood flow and oxygen kinetics to PTF that have been documented in non-septic individuals.

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CARDIOVASCULAR EFFECTS OF PENTOXIFYLLINE IN SEPTIC SYNDROME.

H. Steltzer, N. Mayer, C. Oismüller, J. Winternitz, C. Weinstabl, P. Germann and A.F. Hammerle (Spon: G. Schlag).
Dept. Anesthesia and General Intensive Care, Sepsis Research Group - University of Vienna, A-1090 Vienna

Pentoxifylline (PTX) might have beneficial effects in septic syndrome. While PTX induces increases of cardiac output and decreases of systemic vascular resistance in non-septic patients (1), the cardiovascular responses to PTX in septic states are less well defined. Therfore, we examined the hemodynamic changes after PTX administration in septic patients. **METHODS:** Pulmonary artery catheters were inserted in temporarily six mechanically ventilated patients of either sex who fulfilled established sepsis criteria. After approval by the institutional Ethics Committee, PTX (5mg/kg) was administered intravenously over a period of 3 hrs. Cardiac index, systemic vascular resistance, heart rate, mean arterial pressure, mean arterial pulmonary pressure and stroke volume were recorded before, during and after the PTX infusion. Statistical analysis was performed by ANOVA. **RESULTS:** Cardiac index increased slightly immediately following PTX administration (4.05±0.3, control and 4.95±0.5 l/min, after 30 min, n.s.) and returned to control levels thereafter while systemic vascular resistance index decreased insignificantly from 1358±133 to 1147±83 dyn.sec/cm^5 and remained at this level throughout the entire protocol. There were also no significant changes of mean arterial pressure and heart rate. In addition PTX is devoid of any effects on the pulmonary vasculature. **CONCLUSIONS:** In contrast to findings from non-septic individuals (1) and from results obtained during ß-receptor stimulation, PTX exerts no significant cardiovascular effects in septic patients. Thus the beneficial effects of PTX in experimentally induced septic states (2) are not accompanied by a hemodynamic profile, which by itself characterizes the hyperdynamic cardiovascular response to septicemia in humans.

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IN VIVO MODULATION OF HUMAN NEUTROPHIL FUNCTION BY PENTOXIFYLLIN IN SEPTIC PATIENTS.

C. Oismüller, N.Mayer, H.Steltzer, W.Macheiner, M.Micksche and A.F.Hammerle (Spon:G.Schlag).
Sepsis Research Group, Dep. of Anesthesia and Intensive Care Medicine and Institute for Applied and Experimental Cancer Research,University of Vienna, A-1090 Vienna

Pentoxifyllin (PTX) a methylxanthine derivative is generally used for treatment of symptoms of peripheral vascular disease. Furthermore PTX is able to modulate neutrophil function in vitro and especially to inhibit respiratory burst activity (RBA) in human polymorphnuclear granulocytes (PMN). These effects indicate that PTX may be useful in the treatment of granulocyte mediated diseases and symptoms. We therefore studied the in vivo effect of PTX on human PMN of septic patients. Cells were isolated before treatment and two hours after by a two-step dextran sedimentation and sodium metrizoate Ficoll gradient centrifugation. The remaining erythrocytes were removed by hypotonic lysis. RBA was measured in a chemiluminiscence assay after stimulation with FMLP(formyl-methyl-leucyl-phenylalanin), PMA(phorbol-myristat-acetat) and opsonized zymosan.In this study we demonstrate that PMN of patients with septic syndrome have increased capability for oxydative response to variety of stimuli. We found that PTX i.v. treatment was able to reduce this reactivity when samples were investigated before and two hours after PTX administration.Patients without PTX therapy were found to have unchanged high PMN activity as measured in luminiscence assay. From this study we suggest that PTX therapy is able to modulate granulocyte function and especially oxygen radicals release in vitro and possible in vivo. However, the clinical effects of PTX therapy,which is non toxic in the doses used, in patients with septicaemia have to be further evaluated.

S22: Free Radical Scavengers

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THE EFFECT OF SHOCK ON BLOOD OXIDATION-REDUCTION POTENTIAL.

Arthur E. Baue, Max Jellinek, Bhugol Chandel, Rishart Abdulla, Marc J. Shaprio.
St. Louis Univ. Med. Ctr., 3635 Vista Avenue at Grand Boulevard, P.O. Box 15250, St. Louis, MO 63110-0250.

In hypovolemic shock, hormone activated metabolism mediated through cyclic AMP may generate NADH beyond the oxygen available with declining organ perfusion. From this, sulfhydryls may appear in blood and be detected by oxidation-reduction (redox) potential measurements. Continued saturation of such hydrogen carriers may result in altered transport, receptor configuration and vascular tone by proteins modified through exposure to excess sulfhydryls along with oxyl radical production and 5'nucleotidase stimulation. Redox measurements were made in the blood of rabbits subjected to shock and treated with a mild oxidizing agent (albumin). Normal potential readings corrected for pH were -8.8 ± 1.3 millivolts (mv) arterial blood (A) and -18.0 ± 2.0 mv venous blood (V). A 20 mv drop on the V and a 13 mv on the A side was seen after shock. This did not fully return to normal two hours after volume replacement.

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Infusion of 2 g of albumin/kg/hr raised the V redox potential to normal but it returned to untreated level when albumin was discontinued. It appeared that hydrogen equivalents coming from muscle and organs were partially removed in the lungs. The redox load imposed on the animal by shock appeared to be large and not readily reversed by reperfusion or by the quantity of albumin given. Thus cellular respiration may not be adequately restored and the redox load could impede recovery.

402 ANTIOXIDANT TREATMENT OF INFLAMMATORY TISSUE INJURY.

G.O. Till, University of Michigan Medical School, Ann Arbor, MI 48109

Oxygen-derived free radicals are increasingly recognized for their contribution to inflammatory cell and tissue injury. Experimental studies in our laboratory have demonstrated that oxygen radicals (most likely the hydroxyl radical) derived from neutrophils are involved in the pathogenesis of acute lung injury either caused by systemic complement activation, deposition of IgG immune complexes or thermal injury of skin. Various antioxidants, when applied to these rat models, resulted in a significant attenuation of pulmonary injury. The use of long-lived polyethylene glycol derivatives of both catalase and superoxide dismutase was particular effective, as were iron chelators (deferoxamine, lactoferrin) and scavengers of the hydroxyl radical (dimethyl sulfoxide, dimethylthiourea, dihydroxybenzoic acid, etc.). Recent obser-vations indicated that xanthine oxidase-derived oxygen radicals participate not only in ischemia-reperfuion injury of organ systems such as the intestine and the myocardium but are also playing an important role in the pathogenesis of early burn wound edema as well as acute lung injury subsequent to systemic complement activation. Accordingly, treatment with xanthine oxidase inhibitors (allopurinol, lodoxamide) significantly attenuated acute microvascular injury in both lungs and skin of rats following systemic complement activation and thermal skin injury, respectively. Protective effects of antioxidants were also observed in thermally injured patients who were injected with polyethylene glycol-modified superoxide dismutase. The treatment resulted in a significant reduction in plasma levels of lipid peroxidation products (conjugated dienes). It is hoped that these results will further the use of antioxidants in the treatment of inflammatory tissue injury in human patients.

403 THERAPEUTIC INTERVENTIONS - SEPSIS AND ORGAN FAILURE FREE RADICAL SCAVENGERS.

H.P. Friedl and O. Trentz, Department Chirurgie, Klinik für Unfallchirurgie, Universitätsspital, CH-8091 Zürich, Switzerland

It is well accepted that activation of neutrophils by chemotactic or phagocytic stimuli can dramatically increase their oxygen consumption and result in the production of highly reactive oxygen metabolites, including superoxide anion, hydrogen peroxide and others. In this context the hydroxyl radical may be the most important oxidant involved.

Experimental evidence that oxygen radicals are participating in the pathogenesis of acute pulmonary failure has largely been derived from interventional measures including treatment with antioxidant enzymes, radical scavengers, iron chelators and by elimination of inhibition of oxidant sources such as neutrophiles an xanthine oxidase.

In particular, significant protective effects of hydroxyl radical scavengers have been observed in different neutrophil-mediated acute inflammatory responses accompanied by tissue injury.

Some of these antioxidant compounds and means, that have been employed as tools or probes to elucidate the in- vivo role of oxidants may potentially be used in the treatment of acute pulmonary failure in man. The purpose of the current presentation is to review our findings in animal models of acute lung injury and to discuss potential clinical applications.

404 FREE RADICAL ABLATION FOR THE TREATMENT OF REPERFUSION INJURY: AN OVERVIEW OF RESULTS IN ANIMAL MODELS AND CLINICAL TRIALS.

Gregory B. Bulkley.
The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Superoxide free radicals generated from activated xanthine oxidase at reperfusion trigger a cascade of toxic oxygen metabolites and, in some cases, neutrophil activation that produce a significant proportion of the injury sustained as a consequence of ischemia in many organs. The near-ubiquity of this mechanism appears to be explained by high levels of xanthine oxidase in the microvascular endothelium of most human organs (including the heart and brain). The importance of this reperfusion mechanism, and the potential clinical impact of free radical ablation for the treatment of post-ischemic injury, was

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quantitated to be variable, but often substantial, following varying periods of warm, cold, or warm plus cold renal ischemia in a porcine model of cadaveric kidney transplantation. This study revealed a limited, but substantial "therapeutic window" of ischemia time, following which free radical ablation was successful. A prospective, randomized, double-blind, placebo-controlled, paired, clinical trial of 100 human cadaveric kidney transplantations treated with intra-arterial superoxide dismutase at reperfusion confirmed the presence of, and quantitated the size of this window, and therefore represents the first quantitation of the impact of reperfusion injury, and of the effectiveness of its treatment by free radical ablation, in man. A subsequent, similarly-designed, but multicenter trial of 400 patients showed striking reduction in both post-transplant renal failure and death in those patients receiving kidneys preserved in allopurinol to block subsequent superoxide generation by xanthine oxidase. Results of these as well as of other trials not only demonstrate the importance of the xanthine oxidase free radical-generating mechanism to post-ischemic injury in man, but also reveal variable patterns of applicability in different organ systems, including the kidney, heart, and CNS. These patterns allow the construction of a paradigm for quantitation of the clinical impact of this approach. Clinical trials that naively fail (or have failed) to consider the effect of patient heterogeneity on results because of these limited therapeutic windows are unlikely to be enlightening.

RECOMBINANT HUMAN SUPEROXIDE DISMUTASE (rh-SOD) TO REDUCE MULTIPLE ORGAN FAILURE AFTER TRAUMA - RESULTS OF A PROSPECTIVE CLINICAL TRIAL. 405

I. Marzi, V. Bühren, A. Schüttler[§*], and O. Trentz. Dept. of Trauma Surgery, University of Saarland, D-6650 Homburg/S., and [§]Grünenthal Research Center, D-5100 Aachen, FRG.

Reduction of reperfusion injury by oxygen radical scavengers following ischemia has been demonstrated in a variety of experimental models. However, no data are available on the efficacy of radical scavengers to reduce postraumatic reperfusion injury in the clinical situation. Therefore, the purpose of this study was to evaluate, if the scavenger rh-SOD can reduce the development of multiple organ failure (MOF) in severely polytraumatized patients. 24 patients with an AIS-ISS score $\geq$ 27 were treated with either rh-SOD (3 g/day for 5 days, Grünenthal, Aachen, FRG) or placebo in a blinded, randomized, prospective study. During a 14 days observation period, MOF scores (Goris et al., Arch.Surg. 1985;120:1109), routine clinical and laboratory parameters as well as markers of the inflammatory response (e.g., TNFα, IL-6, C-reactive protein, PMN-elastase, neopterin, phospholipase A, endotoxin, complement) were determined. Both groups were comparable in respect to the initial injury with a median AIS-ISS score of 34 in both groups. MOF scores decreased earlier in rh-SOD group than in placebo group (e.g., at the end of treatment period: 2.8 + 0.5 vs. 3.5 + 0.4; mean + SE). Most inflammatory parameters were decreased in rh-SOD group: e.g. PMN-elastase at day 6 was 57.9 + 5.7 vs. 112.9 + 23.7 µg/l and C-reactive protein was 104 + 16 vs. 160 + 19 mg/l, $p<0.05$). Throughout the study, no drug related side-effects were observed. The decrease of MOF scores as well as the reduction of inflammatory mediators reveal that treatment with rh-SOD may be beneficial to reduce postraumatic organ failure. The preliminary results of this pilot study, however, need further confirmation in a larger number of patients.

THE EFFECTS OF HUMAN TYPE SUPEROXIDE DISMUTASE ON THE SURVIVAL RATE OF ANIMALS WITH VARIOUS TYPES OF SHOCK. 406

R. Ogawa, H. Bitoh, Y. Ohi,.
Dept. of Anesthesiology, Nippon Medical School, Tokyo 113 Japan

It is well known that shock states induce severe injury in various organs. There is increasing evidence that oxygen free radicals (OFR) generated from xanthine oxidase in the ischemic cells and/or NAD(P)H oxidase in the activated phagocytes are responsible for these injuries. If the excessive presence of OFR can be effectively eliminated by any measures, the lipid peroxidation and the insuing cellular injuries would be prevented in animal models.
Wister strain rats were subjected to hemorrhagic, endotoxemic and splanchnic ischemic shock. The effects of various measures to prevent the accumulation of OFR in the organs were investigated. The preventive measures were pretreatment of allopurinol, exogenous supply of human type superoxide dismutase (SOD) and catalase, and administration of chemical quenchers such as -tocopherol and reduced glutathione.
Treatment of 2 mg/100gBW of human SOD produced significant improvement in survival rate in models with endotoxemic and splanchnic ischemic shock, exhitting lesser organ damages and better hemodynamics. The results suggest the possible introduction of human SOD into clinical trial as an effective scavenger of OFR.

EFFECTS OF SOD ON INHIBITION OF HEPATIC DAMAGE INDUCED BY ENDOTOXIN 407

H.Shimada,A.Murai,T.Tsuchiyama,Y.Takahashi,G.Nakagawara
First Department of Surgery,Fukui Medical School,Fukui 910-11,Japan

Eicosanoid mediator which include prostaglandins,thromboxanes,and oxygen radicals are generated during endotoxemia and human gram-negative sepsis and may be important in shock, inducing DIC or hepatic damage in this syndrome.We demonstrated endotoxin-induced DIC and

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hepatic damage using a generalized shwartzman reaction, and assesed the effect of thromboxane inhibitor, prostaglandin I_2 and oxygen radical scavenger on inducing DIC and hepatic damage.

Endotoxin (E.coli 0111 B_4 0.5mg/kg iv) was administrated to 19 adult male dogs. First a preparatory injection was given and the same dose was given as a provocative injection 24 hrs later. The dogs were divided into 4 groups as follows : Control group (n=6), OKY group (n=4) in which OKY 046 of thromboxane synthetase inhibitor was injected (5mg/kg iv) before the preparatory injection, OP group (n=4) in which OP 41483 of PGI_2 derivatives was injected (1μ/kg iv), and SOD (superoxide dismutase) group (n=5) in which SOD of oxygen radical scavengers were injected (30,000 U/kg).

The level of serum complement (C_3) in all groups decreased markedly after the preparatory injection of endotoxin. This fall was prevented slightly by SOD treatment. The increase of leucocyte counts after the preparatory injection was prevented slightly by SOD treatment as compared with other groups. The platelet counts in all groups continued to decrease after the preparatory and provocative injections. The effect of SOD on preventing thrombocytopenia was insignificant. The plasma FDP in all groups increased after the injection. This increase was inhibited by OKY 046, OP41483 and SOD treatment. The 6-keto-$PGF1\alpha$/TXB_2 ratio in SOD treatment stayed above one after the injections, although in other groups it remained less than one after the prepratory injection. The electron micrograph showed that there was less tendency for degeneration of the hepatocyte nucleus and hepatocyte fatty change, and there were no findings of sinusoidal endothelium detachment, globular change of erythrocyte and fibrin in sinusoid in the SOD groups, as compared to the control and other groups.

In this study SOD prevented the complement consumption with leucocytosis, and protected against intravascular coagulation and hepatic damage, however thromboxane synthetase inhibitor and PGI_2 derivatives did not. This data demonstrated that the circulatory collapse induced by ablation of the endothel made by oxygen radicals released from activated leucocytes by the activated complement was an important factor in the hepatic damage induced by endotoxin.

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THE USE OF GLUTATHION IN PATIENTS UNDER SHOCK AND STRESS CONDITIONS

O.Ortolani, M.Pezza, E.Gravino, V.Parlato and R.Tufano Univ.Naples, Anesthesiology and Intensive Care Dept. Via S Pansini 5 80131 Naples Italy.

Stress and shock conditions produce adrenergic, coagulative and complement activation. Hypoxic situations are present in many enzymatic systems. The phospholipase is stimulated and the arachidonic acid cascade produces prostaglandins, thromboxanes and leukotrienes. Of main importance has recently proved the role of granulocyte hyperproduction of free radicals, kinins and interleukins. Antioxidant drugs may reduce many of these unwanted reactions. The present trial was performed on 100 subjects with: 1) Positive C5a activation, 2) Free radical hyperproduction, 3) Presence of hypercoagulative conditions. All the patients were from major surgery (thoracic, cardiac, vascular) and intensive care (severe body lesions with sepsis). Two groups of 50 were randomized for age, sex and pathologies. Both groups received common medical therapies, one group received also glutathion (GSH) 1200 mg/day. The trial lasted 15 days and the treated group showed a marked reduction in the activated fraction of complement (C5a), Ethane in the expirate (Eth), serum malondialdehyde (MDA), and a-Fibrinopeptide (FPA) when compared with the control group. We concluded that GSH is very useful in preventing oxidative damages in patients under shock or in critical conditions.

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SENSITISATION OF SPONTANEOUSLY BEATING NEONATAL RAT CARDIOMYOCYTES TO OXYGEN FREE RADICALS BY DEPLETION OF GLUTATHIONE

U. Müller*, C. Greger*, S. Hallström[1], B. Wagenknecht*, W. Fürst*[1], R. Issels*[2], G. Schleg[1], K. Werdan

Departments of Medicine I and III[2] of the University of Munich, Klinikum Großhadern, Munich, Ludwig-Boltzmann-Institute for Experimental and Clinical Traumatology[1], Vienna

In spontaneously beating neonatal rat cardiomyocytes, oxygen free radicals produced in vitro by the xanthine/xanthine oxidase reaction (0.8 mM xanthine + 50 mU xanthine oxidase) lead to a decrease in cellular contents of reduced glutathione (22.74 ± 2.38 vs. 7.65 ± 0.83 nmol/mg protein) and an increase in the percentage of oxidised glutathione (as GSH-equivalent) (2.71 ± 0.98 vs. 54.40) prior to a fall in ATP- and potassium contents as well as arrhythmogenesis (from 10 min) and standstill (30 min) of contractions. In order to establish a model to test the protective action of reduced glutathione against oxygen free radicals, neonatal rat cardiomyocytes were depleted of glutathione (22.74 ± 2.38 vs. 2.32 ± 0.01 nmol/mg protein) by a 24-hour-incubation with 1mM buthionine sulfoximine, an inhibitor of the γ-glutamyl-cysteinyl-synthestase: the depletion of glutathione is without effect on the ATP/ADP-ratio (10.54 vs. 10.11) and the spontaneous activity of the cardiomyocytes. The depletion of cellular glutathione leads to a sensitisation against oxygen free radicals: arrhythmias (from 5 min) as well as a standstill of the cells (from 20 min) and the oxygen-radical induced fall in potassium contents (600 vs. 400 nmol/mg protein after 30 min) occur earlier; the decrease in reduced glutathione is even more pronounced.

Conclusions: (1) A reduction of the reduced glutathione to 10% of the control value even after 24 hours does not impair the spontaneous activity, the ATP/ADP-ration and the potassium contents of neonatal rat cardiomyocytes. (2) The depletion of glutathione is not primarily responsible for the oxygen-radical induced reduction of contractility. (3) The sensitisation of rat heart muscle cells by BSO documents the protective effect of glutathione towards oxgen free radicals.

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ROLE OF IRON IN REPERFUSION INJURY. Bo Hedlund and Philip Hallaway*, Biomedical Frontiers, Inc. Minneapolis, MN 55414

Trace amounts of "free" or toxic iron are an important, but often overlooked, component in the genesis of free radical mediated tissue injury occurring secondary to ischemia and reperfusion. Although iron is highly sequestered under normal physiological conditions, it has been demonstrated that significant quantities of iron are transiently released during severe ischemic insults, probably via reduction of ferritin bound iron by superoxide and other reducing compounds. Mazur et al. (J. Biol. Chem. 213, 147 (1955) demonstrated that iron in quantities sufficient to fully saturate transferrin binding capacity is released during severe hypotension. In more recent studies, this type of "iron translocation" has also been documented in isolated organs, e.g. the kidney and the intestine, following exposure to an ischemic insult. Iron chelation has been incorporated in many preclinical studies to assess the role of iron in mediating reperfusion injury. Deferoxamine (DFO), the most widely used iron chelator, is reasonably safe and neutralizes iron very effectively and has afforded protection in many of these models. However, the drug is not well suited for parenteral use due to its hypotensive effect and short vascular half-life. The recent development of new forms of DFO based on attachment of the drug to colloids, such as hydroxyethyl starch, eliminates these drawbacks. A colloid-DFO conjugate has been incorporated as an integral part of resuscitation in preclinical studies of burn injury and hemorrhagic shock. Volume replacement with this solution achieves both tissue reoxygenation and systemic, high dose, anti-oxidant therapy. Results from these studies indicate that resuscitation with the colloid-DFO conjugate provides superior attenuation of functional and biochemical indices of target organ dysfunction compared to colloid vehicle alone.

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DESFERRIOXAMINE MESYLATE (DESFERAL) REDUCES SEVERITY OF HISTOPATHOLOGICAL CHANGES IN THE LUNGS OF HAEMORRHAGIC SHOCK DOGS. S.Sanan, D.P.Sanan*, G.Sharma*, J.Rai*, B.Singh*, R.J.Singh* and P.Wadhera*.

Depts: Pharmacology, Surg. and Path. Med.Coll. Amritsar India (Study carried out at*)

Iron catalyses oxy-free radical (FR) production and there is growing evidence for their role in organ failure in shock states. Effect of desferal, an iron chelator, on oligaemic and post-oligaemic histopathological (HP) changes in the lungs (by light microscopy) in anaesthetized dogs has been studied in standard and clinical haemorrhagic shock (HS) models. For oligaemic HP changes, desferal 25 mg/kg i.m. (n=10) was given 30 min after initial rapid arterial bleeding (IB) to MAP 35 mmHg and dogs sacrificed and lungs removed after 4 h. For post-oligaemic studies drug was given at 4, 2+4 h (n=6) and withdrawn blood returned (ROWB) at 4 h after IB ; dogs sacrificed and lungs removed 2 h thereafter. 72 h survival with the same drug regime (n=6 each gp) was investigated in standard HS. For serum iron estimation (Ramsay method) blood samples were taken before IB and 2 h after ROWB in the post-oligaemic experiments. Drug effects were evaluated against parallel untreated control groups for each study.

Desferal was observed to reduce occurrence and severity of oligaemic and post-oligaemic HP changes of lungs in both HS models (scoring done in equiv.microscope fields of drug treated and control gps), and also reduced serum iron elevation observed in shock as compared to the controls ($P < 0.05$- < 0.001). These findings along with increased survival rate obtained with this drug favours the concept envisaged,that decompartmentalized iron in circulatory shock state plays a vital, possibly multifocal, role in initiating and perpetuating free radical mediated organ damage and that desferal is a promising therapeutic possibility for minimising the pathological changes and their sequele.

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DESFERRIOXAMINE INDUCES HYPOTENSION IN EXPERIMENTAL GRAM-NEGATIVE SEPTICEMIA. R.A. Mustard, J. Bohnen, J.B. Mullen, B.D. Schouten, H.T. Swanson (Spon: W. Sibbald) University of Toronto, Wellesley Hospital, Toronto, Canada. M4Y 1J3.

Multiple organ system failure may result from tissue damage caused by activated neutrophils or endotoxin. A significant part of this tissue damage is due to peroxidation induced by oxygen-free radicals and requires iron as a co-factor. Iron chelation has been shown to prevent tissue damage in some models. This experiment was carried out to determine whether iron chelation with Desferrioxamine would prevent lung damage in a swine model of gram-negative septicemia. Fifteen animals were randomized to control, Pseudomonas aeruginosa infusion at a rate of 2×10^7 colony forming unit/20 kg/min. (septic group), or Pseudomonas infusion combined with Desferrioxamine pretreatment at a dose of 80 mg/kg/hr (septic-treated group). Three of six septic-treated animals became severely hypotensive and died during the course of the experiment as opposed to 0 of 6 septic animals. Surviving septic-treated animals were significantly hypotensive (60 $\pm$ 24 mmHg mean arterial pressure) compared to septic (122 $\pm$ 9 mmHg) and control (109 $\pm$ 7.8 mmHg) animals. Desferrioxamine did not improve respiratory function or morphology in septic animals. We conclude that iron-chelation therapy with Desferrioxamine at the above dosage results in a significant deterioration in cardiovascular function in septic swine. Lung damage was not prevented.

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EVALUATION OF IN VIVO FREE RADICAL ACTIVITY DURING ENDOTOXIN SHOCK USING SCAVENGERS, ELECTRON MICROSCOPY, SPIN TRAPS, AND ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

Michael F. Wilson and Daniel J. Brackett, Veterans Affairs Medical Center and Departments of Medicine and Surgery, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, 73104.

Studies conducted in our laboratories provide evidence that excessive free radical generation may be a significant factor in organ damage induced by endotoxin (ETX). Electron paramagnetic resonance spectroscopy combined with spin trapping techniques produced spectra that indicate free radical activity in the heart and liver 25 min, but not 5 min, after ETX. The hyperfine splitting constants of the spin adducts suggest carbon centered radicals of the type produced during lipid peroxidation. Electron microscopy demonstrated damage to the heart and liver 4 hrs after endotoxin challenge and small intestinal hemorrhage and mucosal villi damage has been documented by direct observation and light microscopy. Dimethyl sulfoxide (DMSO) blocked the damage to all three of these organs. DMSO is a hydroxyl radical scavenger, suppresses the production of superoxide anions and hydrogen peroxide by activated neutrophils, inhibits leukocyte adherence in the microcirculation, and of utmost importance readily penetrates cell membranes. These attributes of DMSO implicate neutrophils, free radical activity, and possibly the hydroxyl radical as a factor in ETX induced tissue injury. Since iron is a catalyst of hydroxyl radical production the iron chelator desferrioxamine (DFX) was used as an intervention, but had no effect on tissue damage or organ dysfunction due to ETX. However, DFX does not readily cross cell membranes so intracellular hydroxyl radical activity cannot be eliminated. In summary, in vivo free radical activity has been detected in tissue following ETX and ETX-induced tissue damage has been attenuated by an intervention known to interfere with mechanisms of free radical generation. Data derived from the use of DMSO and DFX indicate that the neutrophil may be a key mediator and if the hydroxyl radical is involved its action appears to be intracellular.

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SURVIVAL AFTER HAEMORRHAGIC SHOCK TREATED WITH ALLOPURINOL AND FREE RADICAL CELLS. D. Mannion, G. Fitzpatrick and M. Feeley. Meath/Adelaide Hospitals, & Trinity College Dublin.

The irreversible loss of functional purine base (Cunningham S.K., Kaveney TV. Sur.Res.10: 305-313,1978) and the formation of free radicals have both been suggested as causes of irreversibility following prolonged haemorrhagic shock (McCord JM. N.Engl.J.Med. 312:159-163, 1985). This study was performed to assess the effect of xanthine oxidase inhibition, free radical scavenging, or both, on the twenty four hour survival of dogs, subjected to irreversible haemorrhagic shock. Haemorrhagic shock was induced in twenty anaesthetised dogs. The animals were bled to a mean arterial pressure of 30 mm Hg. This was maintained for four hours or until 20% of the bled volume had been returned. The dogs were allocated to one of four groups: group 1 was control, group 2 were pretreated with allopurinol 50/kg/day for two days, group 3 received free radical scavengers at the time of reperfusion and group 4 received both therapies. There was no significant difference between the groups with regard to blood loss, blood pressure, heart rate, pulmonary artery occlusion pressure, cardiac output or cardiac index. One of the control dogs died during hypotension, the others survived for periods of 3,6,10 and 24 hours after reperfusion. In group 3, two dogs survived 24 hours and the others 4 hours.Four of group 4 survived 24 hours the other died at 16 hours. All the allopurinol pretreated dogs survived twenty four hours. This difference was statistically significant ($p < 0.01$). This study demonstrates the beneficial effect of xanthine oxidase inhibition on survival and suggests that it is due to preservation of functional purine base. It also demonstrates that free radical scavengers are ineffective. Whether this is because free radicals do not cause significant tissue injury in this situation or whether it is because the scavengers used do not reach the site of tissue injury remains uncertain.

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EFFECTS OF COENZYME Q10 ON THE MEDIATOR CASCADE OF SEPSIS. J Lelli, R Drongowski, B Gastman, D Remick, A Coran (Spon:A Coran).
University of Michigan Medical School, Ann Arbor, MI, 48109-0245.

Coenzyme Q10 (CoQ) has been promoted as an effective agent for reducing the deleterious effects of septic shock by acting as an oxygen scavenger and thus stabilizing mitochondrial membranes and by inhibiting the arachidonic acid metabolic pathway and the formation of various prostaglandins.

This study was undertaken to evaluate the effect of CoQ in a live E. coli model of canine septic shock. Group I animals (n=5) received a LD 100 dose of 10^9 live E. coli/kg and were given no further treatment. Group II animals (n=5) received a 20 mg/kg bolus of CoQ without further treatment. Group III animals (n=5) received 20 mg/kg bolus of CoQ 10 minutes prior to the bacterial infusion.

Mean arterial pressure stabilized at 70% of baseline levels ($p<.002$), while cardiac output remained near 50% of baseline ($p<.053$) in Group III (vs. Group I).

The arachidonic acid metabolites, catecholamines and Tumor Necrosis Factor (TNF) were significantly elevated in Groups I and III (vs. Group II). In contrast, Interleukin-6 (IL-6) was lower in Groups II ($p<.01$) and III ($p<.04$) (vs. Group I). Fluorescent Products (Lipid Peroxidation Activity) were elevated in Group I (vs. Groups II and III).

This study demonstrates that CoQ provides hemodynamic stability in live E. coli septic shock. These data also show (in contrast to previous reports) that CoQ does not inhibit

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the production of the catecholamines or the arachidonic acid metabolites but does inhibit the production of IL-6. Since it is known that both endotoxin and TNF are the main mediators of IL-6 formation, it would appear that CoQ exerts its beneficial effects by blocking the cytokine cascade between TNF and IL-6.

XANTHINE OXIDASE ACTIVITY IN A HUMAN MODEL OF ISCHEMIA REPERFUSION INJURY. 416

HP Friedl, J Frank, OA Trentz, GO Till, PA Ward, and O Trentz.
Klinik für Unfallchirurgie, Universitätsspital Zürich, CH-8091 Zürich, University of Michigan Medical School, Department of Pathology, Ann Arbor, Michigan 48109 and Chirurgische Universitätsklinik, D-6650 Homburg.

Oxygen radicals generated by xanthine oxidase activity are implicated in cell and tissue injury resulting from ischemia-reperfusion procedures. We have recently shown in various rat models that histamine modulates in vivo and in vitro the activity of xanthine oxidase. The present studies were designed to investigate if similar mechanisms can be demonstrated following ischemia-reperfusion in man. Patients with upper extremity surgery performed under tourniquet (n=25) were used to study ischemia-reperfusion events. METHODS: Patients for study were selected at random and were treated in compliance with institutional procedures for the study of human subjects. Sequential blood samples were drawn immediately, and 3,5,10,20,30 and 60 min after tourniquet release. Control samples were obtained before and after tourniquet placement. Blood samples were collected in a medium to prevent artifactual xanthine dehydrogenase/xanthine oxidase (XD/XO) conversion. XO activity was determined by measurement of uric acid formation following addition of xanthine +/- NAD+. Histamine levels were assayed by radioimmunoassay. Acute edema formation subsequent to surgical treatment was measured by determination of the circumference of the reperfused and the contralateral limb. RESULTS: Immediately after tourniquet release there was a significant and progressive increase of XO activity peaking within the first 10 min of reperfusion. This was paralled by a 3.4-fold increase in plasma histamine levels; XD activities remained unchanged. Within the observed time course, control samples did not reveal any changes in levels of XD, XO or histamine. Plasma also contained evidence of products consistent with the formation of oxygen free radicals, namely, the appearance predominantly in the reperfused limb of hemoglobin and fluorescent compounds.Edema formation showed a maximum 24 h post tourniquet release. CONCLUSIONS: Our data suggest that ischemia-reperfusion conditions and operative trauma *in humans* can cause in parallel a significant increase of plasma histamine levels and an increase in plasma XO activity independent of a conversion of XD to XO.

INFLUENCE OF rh-SUPEROXIDE DISMUTASE ON INTERLEUKIN-6 PLASMA LEVELS IN POLYTRAUMATIZED PATIENTS. 417

S. Flohé*2, I. Marzi[1], A. Schüttler*3, P.C. Heinrich*2, and V. Bühren[1]. [1]Dept. of Trauma Surgery, Univ. of Saarland, D-6650 Homburg/S., [2]Inst. for Biochemistry, RWTH Aachen, and [3]Research Center, Grünenthal, D-5100 Aachen, FRG.

Interleukin-6 (IL-6), an early mediator of the inflammatory response and stimulator of the acute phase reaction, has been found elevated during sepsis, after major surgery and burns. Beside other factors, endothelial cell injury and microcirculatory disturbances have been proposed to initiate IL-6 release. The aims of this clinical study were 1) to determine IL-6 levels in severely polytraumatized patients, and 2) to evaluate if prevention of oxygen radical mediated injury by treatment with the scavenger recombinant human superoxide dismutase (rh-SOD) can reduce IL-6 levels after trauma. 18 patients with an AIS-ISS score > 27 were randomly assigned either to a treatment (3 g/day rh-SOD, Grünenthal, Aachen, FRG for 5 days) or placebo group. IL-6 was determined daily from plasma samples using the B9-bioassay (Eur.J.Immunol 17:1411,1987). Both groups were comparable in respect to the initial injury as determined by the ISS scores (SOD: 32.6 + 4.2; Placebo: 32.3 + 2.9; mean + SEM). IL-6 levels were similarly elevated in both groups at the begin of therapy, but lower levels were found in the SOD group in contrast to the placebo group during the whole period of therapy:

GROUP	Prior therapy	Day 1	Day 2	Day 5
Placebo	150.1 + 29.7	239.2 + 73.5	342.1 + 117.1	142.3 + 31.9
rh-SOD	129.5 + 36.4	88.7 + 22.9	133.6 + 34.3	96.4 + 51.5

Mean + SEM; pg/ml.

The elevation of IL-6 indicates an early inflammatory response after polytrauma. The efficacy of rh-SOD treatment in reducing IL-6 levels suggests that oxygen free radicals contribute to the release of IL-6 in polytraumatized patients.

rhSOD DOES NOT PREVENT LOSS OF PLASMA ANTIOXIDANTS IN HYPOVOLEMIC-TRAUMATIC SHOCK IN BABOONS. 418

H. Gasser, H. Redl, G. Schlag, K. Radmore* and J. Davies*, Ludwig Boltzmann Inst. Exp. Clin. Traumatol., Vienna, Austria; * Roodeplaat Reserach Lab., Pretoria, South Africa

We hypothetize that loss of plasma antioxidants and formation of lipid peroxides is related to reperfusion injury, which we have tried to attenuate by rhSOD application.

Methods: Our model in EEG-controlled anesthetized baboons involves closed femur fracture, soft tissue injury and hypovolemia (40 mm Hg) over 3 hours, as well as resuscitation over 3 hours (reinfusion: shed blood-Ringer's solution 1+1). In 9 animals rhSOD was infused over 5 hours (6,

Notes

30, 120 mg/kg), starting 30 minutes post trauma. 5 animals were used as controls and 2 as sham animals. Radical release was assessed by measurement of antioxidants (alpha-tocopherol, SH groups) and lipid peroxidation (conj. dienes, TBARS, fluorescence) in plasma. Additionally, bacterial translocation (BT) was investigated using bacteriological techniques.
Results: Radical-related parameters in plasma were not positively influenced by SOD. BT in blood and tissue samples was seen in 7 of 9 animals with SOD and in 4 of 5 animals without SOD, both during shock and after reinfusion.
Conclusion: Despite high doses of continuously applied rhSOD, radical-related plasma parameters were not influenced. This indicates that the action of SOD is ineffective and/or that ischemia rather than reperfusion events are responsible for BT. BT was not affected by this therapeutic regimen.

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SUPEROXIDE DISMUTASE IN COMBINATION WITH AN ANTIBIOTIC IMPROVES THE SURVIVAL RATE OF SEVERE PERITONITIS IN RATS. M.Muramoto, N.Shinagawa, H.Takeyama, M.Taniguchi, T.Hayakawa, K.Katoh, S.Ishikawa, J.Yura and R.Shinohara
First Department of Surgery, Nagoya City University Medical School, Nagoya, Japan

It is well known that in the severe infection the systemic neutrophils are activated and that free radical production is much elevated. And free radical thus radiated in the extracellular space supposedly do harm. Pursuantly, peritonitis was induced in Sprague-Dawley rats with Escherichia coli. Retaining one group as control (n=267), rats were divided into 3 groups. Recombinant human SOD was administered to the SOD group (n=53), while Ceftizoxime Sodium (CZX) was given to the CZX group (n=54). The combination group (n=28) received both CZX and SOD. Each drug was given intraperitoneally 6 hours after peritonitis developed, and then every 12 hours. SOD given in the peritoneal cavity showed a very good pharmacokinetic data both in serum and ascites of the rats with peritonitis. Cumulative survival rates markedly improved for the SOD ($P<0.05$) and CZX ($P<0.001$) groups when compared with controls. The combination group also improved markedly compared with the CZX group ($P<0.05$). SOD activities and lipid peroxide (LPO) levels in liver, lung and serum were assayed with the nitrite and TBA methods, respectively. In 6 hours SOD in serum was induced, but it got much decreased in dead rats when compared with that of surviving rats regardless of the groups. GOT, GPT and BUN in serum were even further elevated in dead rats of all the groups than those of surviving rats. White Blood Cell counts in 3 hours were elevated in all the groups, but in 9 hours they got strikingly decreased as is often observed in the severe infection. The cause of death of this peritonitis is, I suspect, organ failure and acute immunodeficiency due to leukopenia as the result of autolysis of neutrophils, and both of them are related to free radical. In the result SOD administration in combination with a suitable antibiotic improved the survival rates of severe peritonitis in rats.

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IRON CHELATION WITH A DEFEROXAMINE CONJUGATE IN HEMORRHAGIC SHOCK. D.M. Jacobs, J.M. Julsrud*, M.P. Bubrick* Hennepin County Med. Ctr., Minneapolis, MN 55415

Oxygen-derived radicals are cytotoxic, highly reactive molecules that contribute to cellular death and injury in hemorrhagic shock. Iron released into the plasma in hemorrhagic shock may contribute to cellular damage by catalyzing lipid peroxidation of cell membranes. Deferoxamine (DFO) chelation of transitional metal ions events formation of these radicals and may diminish reperfusion injury. Conjugation . DFO to pentastarch (PS) decreases DFO toxicity and extends its half-life making it a potentially useful resuscitative fluid. A porcine hemorrhagic shock model was used to evaluate the effects of 5 resuscitative fluids on survival and hepatic function. Swine (11-16 kg) underwent splenectomy, liver biopsy and placement of arterial and venous catheters. Awake animals were bled at 1 ml/kg/min to a MAP of 45 mmHg, maintained for 1 hour, and resuscitated over 30 mins. with 1 of 5 fluids (all groups n=6): lactated Ringer's (LR); LR + free DFO 2.5 mg/ml (LR+DFO); 5% PS in LR (PS); 5% PS + free DFO (PS+DFO) 7.5 mg/ml; 5% PS/DFO conjugate (7.5 mg/ml) in LR. LR and LR+DFO received 3 ml/ml shed blood; PS, PS+DFO and PS/DFO received 1 ml/ml shed blood. There were no significant differences between groups in MAP, HR, CVP, T or Hct pre and post-resuscitation. No LR animal lived to sacrifice at 24 hours. 33% of LR+DFO and PS+DFO animals died within minutes of receiving the free DFO-containing resuscitative fluid. All other animals survived to sacrifice at 24 hours. Aspartate aminotransferase (AST) levels at 24 hours were significantly lower ($p < .05$) for the PS/DFO animals compared to all other groups; there was no significant difference between the baseline and 24 hour AST level for the PS/DFO group. Malonyldialdehyde (MDA), a marker of lipid peroxidation, was measured in liver homogenates and PS animals showed a 73.5 $\pm$ 32.9% increase from baseline at 24 hours but PS/DFO conjugate animals were essentially identical at baseline and 24 hours (0.1% $\pm$ 16.4%, $p < .0001$). Iron chelation with a deferoxamine-hetastarch conjugate limits lipid peroxidation and may diminish reperfusion injury.

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EFFECT OF DIMETHYLTHIOUREA ON RAT CARDIOPULMONARY RESUSCITATION.
Zi-hui, Xiao and Zheng-yao, Luo. Department of Pathophysiology, Hunan Medical University, Changsha, Hunan, 410078 PRC.
Dimethylthiourea (DMTU) is a scavenger of H2O2 and hydroxyl free radical. The purpose of this study was to determine whether DMTU could improve cardiopulmonary resuscitation (CPR) in rat. The rats were divided randomly into two groups: 1) CPR, 2) CPR + DMTU (165 mg/kg, iv). CPR was induced by massaging the heart and starting the mechanical ventilation after 6 min. cardiopulmonary arrest by injection of 2% cold KCL into left ventricle and discontinuation of mechanical ventilation. The parameters in tab. were measured and the results showed as follows:

Groups	n	Recovery of ECG (min)	Recovery of BP to 80mmHg (min)	Recovery of spontaneous respiration (min)	Catalase activity (u/mg pro.)
CPR	11	9.5±1.5	10.3±1.6	14.5±1.5	0.972±0.047
CPR+DMTU	7	3.9±0.4**	4.5±0.0.7*	8.0±0.6**	1.232±0.045 n=4

*p<0.05, ** p<0.01, Vs CPR group
In conclusion, 1) DMTU could improve cardiopulmonary resuscitation; 2) H2O2 might play an important role in ischemia-reperfusion injury during CPR.

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PROTECTIVE EFFECT OF TUNGSTEN ON RAT CARDIAC INJURY INDUCED BY ENDOTOXIN.
X.Z Xiao*, Z.Y. Luo, Q.M.Zhou*, Q.S.Zhan*, Z.X.Cheng*. Department of Pathophysiology, Hunan Medical University, Changsha, Hunan, 410078, People's Republic of China.

The present study was attempted to observe the protective effects of tungsten(W) on cardiac injury induced by endotoxin in rats and to analyze its mechanisms. 22 male Wistar rats were randomly divided into two groups: 1) ET group (n=11) was given a bolus injection of disintegrated E.Coli (5.4×10^{10} organisms/kg); 2) ET + W group (n=11) was given an equivalent E.Coli after being pretreated with sodium tungstate rich food and water for 3-4 weeks. The results showed that the following parameters in ET + W group had significant difference at 120 minute after injection of ET when compared with ET group ($p < 0.05$ or 0.01): BP(78.2 ± 6.4 vs 31.8 ± 9.9 mmHg), total count of WBC (114.8±9.17% vs 41.0± 4.9%), PMN% (131.9±6.0 vs 41.6±4.7), LVSP(131.4±10.1 vs 60.5±18.2 mmHg), +dp/dt max (5518.2±328.3 vs 2936.4 ± 908.8 mmHg/s), -dp/dt max (3854.6 ± 241.0 vs 2027.3 ± 628.1 mmHg/s), survival rate (100% vs 54.5%), plasma concentration of malondialdehyde (5.9 ± 1.2 vs 11.1 ± 4.4 nmol/ml), activity of catalase in myocardium (23.8 ± 7.4 vs 13.9 ± 7.5 u/mg protein), activity of xanthine oxidase in myocardium (4.76±1.44 vs 14.08 ± 3.08 u/mg protein), the ratio of W/Mo in myocardium (18.50±2.15 vs 0.96±0.22) respectively. Pathologic examination revealed less subendocardial hemorrhage and infiltration of WBCs in ET+W group than in ET group.

In conclusion, tungsten significantly attenuated cardiac injury induced by endotoxin, its mechanism might be related to the inhibition of xanthine oxidase activity by W and the resultant decrease of reactive oxygen species.

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THE PROTECTIVE EFFECT OF DIMETHYLTHIOUREA ON BOVINE PULMONARY ENDOTHELIAL CELLS INJURY INDUCED BY HYDROGEN PEROXIDE
Yanru Wang* Jialu You* Zhengping Han* (Spon. Zhengyao Luo) Dept. of Pathophysiology, Hunan Medical University, Changsha, Hunan 410078, PRC

Recently, a body of evidence demonstrated that reactive oxygen metabolites have been implicated as the initial toxic agents leading to cell damage in pulmonary postischemic reperfusion injury and adult respiratory distress syndrome (ARDS). The purpose of this study was to investigate whether hydrogen peroxide(H_2O_2) could damage cultured bovine pulonary endothelial cells(BPEC) directly and the protective effect of dimethylthiourea(DMTU) on this oxidative injury. Confluent monolayers of BPEC were incubated with H_2O_2 for 1 hr. BPEC were injured with a range of H_2O_2 dose (0~30 mM). The higher concentration of H_2O_2 consistently produced contraction and rounding of >50% of cells. The range of 5~30 mM H_2O_2 decrease plasma membrane fluidity, superoxide dismutase(SOD) and catalase activities and increase the content of malondialdehyde(MDA) of BPEC in a dose-dependent manner. DMTU(20, 30mM) could attenuate H_2O_2 (20mM)-induced damage of BPEC and the higher dose was more effective than lower one. In conclusion: 1) H_2O_2 could exert a direct, dose-dependent cytotoxic effect on BPEC . 2) DMTU had a protective effect against H_2O_2-induced BPEC injury.

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THE PROTECTIVE EFFECT OF DIFFERENT OXYGEN RADICAL SCAVENGERS ON HYPOXIA-REOXYGENATION-INDUCED RAT LUNG INJURY. D. Wang, S.J. Kao, K. Hsu, H.I. Chen
National Defense Medical Center, Taipei, Taiwan, R.O.C.

Reperfusion injury of ischemic or hypoxemic tissue may result from massive free oxygen radicals released during reoxygenation. It has been reported that tissue hypoxia will increase the activity of xanthine oxidase (XO). In order to determine the relationships between XO and oxygen radicals in the injury of isolated perfused rat lungs after reoxygenation, the capillary filtration coefficient (Kf) and uric acid (UA) level were obtained before and after hypoxemia. In addition, the pulmonary vascular hemodynamics were also evaluated. The K_f averaged 15.5 ± 0.8 and 29.1 ± 4.1 $ml.min^{-1}.cm\ H_2O.mg^{-1}$ (N=6) in the control and hypoxia-reoxygenation lungs (H/R), respectively. The increase of the ratio of lung weight to body weight and K_f can be prevented by pretreatment with dimethylthiourea (DMTU)(N=5) or allopurinol (N=6) or superoxide dismutase(SOD) and catalase together (N=6). On the other hand, this increase was not altered by pretreatment with SOD (N= 5) or catalase (N= 6) alone. The UA level was 400% greater in H/R than in control group and this increase was abolished by administration of allopurinol(N=6). The total vascular resistance increased 100% in H/R lungs. Hemodynamic analysis revealed that the major segment involved was the pre-capillary vessel. These results indicate that both hydroxy radical and xanthine oxidase might play important role in the hypoxia-reoxygenation induced lung injury in rat.

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EFFECT OF TUNGSTEN ON THE RAT HEPATIC INJURY INDUCED BY ENDOTOXIN.

Zheng-yao.Luo, Qi-ming.Zhou*, Xian-zhong.Xiao*, Zheng-xiong. Cheng*, Qun-shan.Zhan*. Department of Pathophysiology, Hunan Medical University, Changsha, Hunan, 410078 PRC.

The present study was to determine whether tungsten (W, an inhibitor of xanthine oxidase) could attenuate the rat hepatic injury induced by endotoxin. The rats were divided into two group: 1). endotoxin group; 2). endotoxin +W group: the rats were pretreated with W enrich diet for 3-4 weeks prior experiment. All anethetized rats subjected to a bolus injection of disintegrated E. Coli organisms $5.4x10^{10}$/kg. The parameters in following tab. at 120 minutes after injection were measured.

group	n	BP[1]	WBCs in peripheral blood		liver				mortality
			TC[2] ($x10^3/mm^3$)	PMN%	SGPT (u)	XO (um/mg.Pr)	MDA (nM/mg.Pr)	W/Mo	%
ET	10	37.8 ±9.9	41.6 ±4.8	41.6 ±4.7	119.1 ±21.1	153.8 ±8.2	9.51 ±1.0	0.16 ±0.05	4.5
ET+W	12	78.2 ±6.4**	114.8 ±9.2**	131.9 ±6.0**	59.3 ±7.3*	80.8 ±11.7**	5.33 ±0.2**	6.54 ±0.65**	0*

1) % of baseline 2) total count * P<0.05, ** P<0.01 Vs ET group

Conclusion: 1). W could attenuate the hepatic injury induced by endotoxin; 2). Oxygen Free Radicals derived from xanthine oxidase rather than from PMNs play an inportant role in the pathogensis of endotoxic shock.

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THE PROTECTIVE EFFECT OF ADMINISTERED CoQ_{10} OR α-TOCOPHEROL AGAINST HEPATIC DAMAGE CAUSED BY ENDOTOXEMIA OR ISCHEMIA-REPERFUSION. C.Matsusaka, K.Dohi, S.Marubayashi, K.Sugino, and T.Kawasaki.
Hiroshima University School Of Medicine, 1-2-3 Kasumi Minamiku Hiroshima 734, Japan.

The present study was undertaken to determine whether hepatic damage in cases of endotoxin shock can be explained by lipid peroxidation and whether administration of CoQ_{10} or α-tocopherol (α-toc) can preserve the hepatic functions and thus enhance survival of endotoxin-administerd mice. Intraperitoneal injection of lipopolysaccharide (LPS) to mice at a dose of 15mg/kg of body weight resulted in a survival rate of 31% 48hours after administration. Simultaneous intramuscular administration of (10mg/kg) CoQ_{10} increased the survival rates of LPS-administered mice to 69.7%. When LPS administration was increased to 30mg/kg, no survivors were observed in the placebo group. Simultaneous intravenous injection of CoQ_{10} (10mg/kg) or α-toc (20mg/kg) restored the survival rate to 52.9% or 42.9%, respectively. The adenosine triphosphate (ATP) level in the liver decreased gradually to 70% of the control ATP level 24hours after LPS (15mg/kg) administration. The lipid peroxide level in the liver increased fivefold 16hours after LPS administration and then decreased to the control level in 8hours. Simultaneous treatment of mice with

Notes

antioxidants, such as CoQ_{10} or α-toc, completely suppressed the lipid peroxide level in the liver and preserved the hepatic ATP level in the normal range. The effects of pretreatment of CoQ_{10} and α-toc was also observed in rat hepatic ischemia and subsequent reperfusion. These results indicate that LPS induced hepatic damage in mice because of lipid peroxidation and that antioxidants suppressed lipid peroxidation, preserved energy metabolism in the liver, and enhanced survival of endotoxin-administered mice.

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TIME COURSE OF ISCHEMIA-REPERFUSION INDUCED OXYGEN FREE RADICAL GENERATION IN THE LIVER: RELEVANCE TO MANAGEMENT OF SHOCK. M. Okuda*, I. Ikai, B. Chance and C. Kumar*.
Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6089 and Institute of Biophysical and Biomedical Research, Philadelphia, PA 19104, USA.

While the role of oxygen radicals in ischemia-reperfusion induced tissue injury is widely accepted, no methods were available for the direct measurement of tissue oxygen radical concentrations until recently. We have now applied the Luminol enhanced chemiluminescence (LEC) method to monitor ischemia-reperfusion induced oxygen radical production in heart and liver quantitatively and continuously. The LEC intensity under these condition is a direct measure of the instantaneous oxygen radical concentration in the tissue. Livers were subjected to 2 x 30 min or 1 x 60 min of ischemia, followed by reperfusion. At each reperfusion, there was a transient and intense burst of oxygen radical production (Response A). On reperfusion after 60 min of cumulative ischemia, there was a continuous, sustained increase in tissue oxygen radical production that persisted for several hours (Response B). We also studied the effect of free radical scavengers (SOD, catalase and allopurinol) on the time course of oxygen radical production in liver during ischemia-reperfusion. SOD administration abolished Response A and attenuated Response B. Catalase administration enhanced Response A and attenuated Response B. Allopurinol did not affect Response A, but attenuated Response B. The implications of these first direct measurements of oxygen radical production in the liver and allied biochemical studies for the management of shock will be discussed; it is clear that, to be useful, free radical scavengers should be effective against both Response A and Response B, and should be administered long enough following reperfusion in order to abolish Response B completely.

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THE EFFECT OF MDTQ-DA (GROUP 1) AND MDTQ-DA IN COMBINATION WITH TRASYLOL AND ASCORBIC ACID (GROUP 2) WAS STUDIED IN VIVO ON PIGS WITH EXPERIMENTALLY INDUCED PANCREATITIS. T. Zimmermann, S. Albrecht, S. Koppraseh and H. Kühne
Medical Academy Dresden, Dresden, O-8019, Germany

Evaluation parameters:
1. *CL-response of granulocytes and lymphocytes/monocytes in whole blood*
2. serum changes of the kallikrein-kinin-system (KKS)
3. liver enzymes
4. glutathione (GSH-GSSG) status of the organs

Results:
Group 1: Reduction in CL-response by 50-70 p.c. Inhibition of the inflammatory reaction. No influence on the pancreatogenic shock
Group 2: Reduction in CL-response by 70-80 p.c. Inhibition of the KKS. Prevention of MOF and pancreatogenic shock. Improvement of the glutathion status of the organs.

The combined therapy of acute pancreatitis with radical traps, kallikrein inhibitors and natural antioxidative substances is capable of preventing the development of the MOF.

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OXYGEN FREE RADICAL-INDUCED CELL INJURY IN RAT HEART MUSCLE CELLS
B. Wagenknecht*, M. Hug*, C. Freudenrich*[1], S. Hallström[2], G. Hübner*[3], M. Lieberman*[1], G. Schlag[2], K. Werdan
Dept. of Medicine I, Klinikum Grosshadern, [3]Inst.of Pathology, University of Munich, F.R.G.; [2]Ludwig-Boltzmann-Inst. f. Exp. u. Klin. Traumatologie, Wien, Austria; [1]Dept. of Cell Biology, Duke University Med. Center, Durham, USA
Oxygen free radicals (OFR) are mediators, responsible for myocardial contractile dysfunction during shock and ischemia/reperfusion. To study the direct effects of OFR in cardiomyocytes, we exposed neonatal rat heart muscle cells to 0.8 mM xanthine + 50 mU xanthine oxidase for up to 75 min. This OFR-generating system produces superoxide radicals (initial rate: 13 nmol/min), hydrogen peroxide (final conc.: 400 μM) and hydroxyl radicals in a time dependent way. Negative chronotropic and arrhythmogenic effects ($T_{1/2}$: 15 min) correlate with a decrease of GSH/GSSG ratio (t=0: 11 %, t=15 min: 68 % GSSG). The cardiotoxic effects are characterized by a decrease of ATP-content

Notes

($T_{1/2}$:20 min) to 15%; K-content is reduced by 55% ($T_{1/2}$:35 min), Na-content increases 8-fold ($T_{1/2}$: 40min); Na/K-ATPase-mediated $^{86}Rb^+$ uptake is decreased to 10% ($T_{1/2}$:40 min) followed by a 4-fold increase of cytosolic free calcium up to 1200 nM ($T_{1/2}$: 55min) and ultrastructural alterations (swelling of mitochondria, hypercontracture of myofibrils). Catalase (100 U/ml) but not SOD (100 U/ml) protects against the cardiodepressive as well as cardiotoxic effects of OFR.
Conclusion: 1. OFR-induced ionic alterations are due to inhibition of active Na^+/K^+-transport and are not a result of unspecific membrane damage. 2. Impairment of contractile function precedes the increase in cytosolic free calcium, the latter being responsible for OFR-induced cardiotoxicity. 3. In this experimental model system hydrogen peroxide but not superoxide radicals seem to be the dominant damaging free radical species.

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DIRECT EVIDENCE OF FREE OXYGEN RADICALS IN REPERFUSION INJURY OF RAT LIVER BY ELECTRON SPIN RESONANCE. R. Kunz, H. A. Brune*, H. G. Beger. Departments of General Surgery and *Organic Chemistry I, University of Ulm, Steinhoevelstr. 9, 7900 Ulm, Germany
Increased generation of free oxygen radicals as a main pathogenetic principle of reperfusion injury could be demonstrated in small intestine, heart muscle and brain tissue. Radical mediated reperfusion injury of liver tissue is still under discussion as long as free radicals could not be detected directly so far.
Material and Methods: The experiments were performed with male Wistar rats in fentanyl/fluanison-diazepam anesthesia. Before, during and after 60 min. ischemia of the left lateral and median liver lobe the tissue was freeze-clamped. For ESR studies Phenyl-N-tert-butyl-nitrone (PBN) (Sigma, Munich) was used and PBN spin adducts monitored in a Varian E 4 ESR spectrometer after extraction into ethylacetate.
Results: Spectra of radical adducts could be obtained after administration of PBN as spin trap. In vivo control experiments demonstrated a decline of spectrum intensity as a sign of reduction of PBN-radical adduct in vivo. In contrast to these results after liver ischemia of 60 min. the ESR signal did not decrease during reperfusion but increased, at least for 45 min. of reperfusion time representing an increased generation of free radicals in this period. A maximum of radical production could be observed after 15 min. of reperfusion time. The measured hyperfine splitting constants of the obtained PMB/radical adducts were identical to known values of PBN/ OH and PBN/ OOH. In additional experiments reperfusion injury could be avoided by administration of deferoxamine and superoxyde dismutase/catalase but not by allopurinol.
Discussion: For the first time free radicals could be monitored directly in rat liver reperfusion injury by electron spin resonance. The effect of deferoxamine SOD/catalase proved, that reperfusion injury is hydroxyl-radical-mediated. The conversion of xanthine dehydrogenase to oxidase is unimportant because allopurinol had no effect.

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ROLE OF FREE RADICALS IN THE REPERFUSION INJURY OF RED AND WHITE MUSCLES IN THE RAT.
L. EGRI, J. HAMAR, Z. DEMEL, K. KÁNTÁS, J. RUPNIK and E. PURUCKER, A.J. AUGUSTIN, J. LUTZ.
National Institut of Traumatology, Budapest, Hungary, and Department of Physiology University of Würzburg, Germany.

A 4 hour tourniquet ischemia was applied on the left leg of anesthetized rats. The right leg served as control. After the release of the ligature the legs were allowed to be reperfused for 0, 1, 2, 4, and 12 hours. The extensor digitorum longus (EDL) and soleus muscles (SM) as white and red muscles were removed for nalyses. Thiobarbituric acid reactive substances (TBARS) as a measure of free radical content were detemined. Tissue concentrations of the oxidized (GSSG) and reduced (GSH) forms of glutathion as an indicator of naturally occuring defence mechanisms were also measured. Tissue concentrations of GSH and GSSG and also TBARS were higher in the SM than in the EDL in control. 4 hours of ischemia resulted in an increase of 16 %, 32 %, 45 % (ED)L and 19 %, 49 %, 42 % (SM) in the GSH, GSSG, and TBARS concentrations, respectively. GSSG/GSH ratio was unchanged. GSH decreased within 2 hours of reperfusion in both muscles, TBARS increased at the same time and GSSG/GSH was also higher. No recovery of the parameters was observed up to 12 hours. The increase in TBARS was secondary to the depletion of cellular antioxidants. Antioxidant treatment by methyl-prednisolon, deferoxamine and SOD-catalse did not prevent GSH decrease, however, GSSG/GSH was close to normal and the increase in TBARS was also reduced.

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ROLE OF HISTAMINE IN INTESTINAL REACTIVE HYPEREMIA FOLLOWING MESENTERIC ISCHEMIA. J. Kaszaki*, M. Boros* and S. Nagy
Szent-Györgyi Albert Medical University, H-6701 Szeged, Hungary.
Sudden reperfusion of the gut following prolonged ischemia can in itself be more deleterious than ischemia alone. In recent studies we demonstrated a link between the release of histamine (H) and reactive oxygen intermediates (ROI) generated by xanthine oxidase (XO) during reperfusion

Notes

of a previously ischemic canine ileal segment. In the present study we examined a possible role of H in the reactive hyperemic response (RH) after a period of total segmental ischemia. The artery supplying the terminal ileum was occluded in anesthetized dogs. An ischemia of 30 min was followed by a 30 min reperfusion period (control reactive hyperemia, CRH) and we measured arterial blood flow to the segment. After CRH one of the following treatments was given i.v.: histamine H_1 or H_2-blockers (tripelennamin 0.5 mg/kg, cimetidine 10 mg/kg, ranitidine 2 mg/kg), the XO blocker allopurinol (50 mg/kg), cromolyn (mast cell stabilizer, 25 mg/kg) and aminoguanidine (a blocker of histaminase, 50 mg/kg). The 30 min ischemia-30 min reperfusion cycle was then repeated (test reactive hyperemia,TRH). Results: Mesenteric RH following 30 min ischemia is reproducible once without a significant change in its parameters. The time integral of blood flow (IBF) during the first 10 min of TRH was significantly decreased by cimetidine, ranitidine, allopurinol, or cromolyn, it was increased by aminoguanidine, while tripelennamin did not affect the post-ischemic vasodilator response. Conclusion: At the onset of reperfusion ROI generated by XO play a role in a release of endogenous H from the gut. The H released participates in causing the postischemic RH through the H_2-receptors.

PROTECTIVE EFFECTS OF ANTIOXIDANTS IN SPLANCHNIC ARTERY OCCLUSION SHOCK

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A. Sakamoto*, S.T. Ohnishi*, T. Ohnishi* and R. Ogawa
Philadelphia Biomedical Research Institute, Pennsylvania, U.S.A. and Dept. of Anesthesiology, Nippon Medical School, Tokyo, Japan.

Ischemia/reperfusion injury has been considered to involve the oxygen-free radical formation and protective effects of antioxidants have been reported in many shock models. In this study, relationships among the free radical formation, the edema formation and the plasma depletion were evaluated. Rats were subjected to 30 min celiac and superior mesenteric arteries occlusion followed by reperfusion. The free radical formation (FRF) of the intestinal mucosa was evaluated by the spin-trapping technique using PBN and electron spin resonance spectroscopy. The lipid peroxidation (LPO) of the intestinal mucosa was evaluated by thiobarbituric acid method. The edema formation was evaluated by measuring the specific gravity (SG) of the intestine. The plasma depletion was evaluated from an increase in the hematocrit (Ht). FRF was already seen during ischemia, and a further increase was seen during the reperfusion period. After 45 min of reperfusion, LPO and Ht increased significantly (LPO: from 0.128 to 0.261 nmols/mg protein; Ht: from 39 to 59 %) and SG decreased significantly (from 1.042 to 1.028). Antioxidative prostaglandin oligomeric derivatives suppressed increases in FRF and in LPO, and inhibited changes in Ht and in SG by 30%. Our results shows that antioxidants have some beneficial effects on the edema formation and the plasma depletion in this shock model.

THE RELATIONSHIP BETWEEN XANTHINE OXIDASE AND HISTAMINE RELEASE DURING INTESTINAL ISCHEMIA-REPERFUSION.

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M.Boros*, J. Kaszaki*, L. Bakó*, S.Nagy.
Inst. of Exptl. Surgery, Szent-Györgyi Albert Medical University and Biol. Res. Ctr. of Hungarian Acad. Sci., H-6701 Szeged, Hungary

In our previous studies we demonstrated that xanthine oxidase (XO)-derived reactive oxygen intermediates are causative agents in histamine (H) release during reperfusion of the ischemic gut. Pretreatment with the XO inhibitor allopurinol resulted in a 87% decrease in the liberated amount of H at the onset of the reperfusion. The role of H in the pathophysiology of intestinal ischemia-reperfusion injury is unclear. Some recent data suggest that H can enhance the activity of XO. To examine this possibility we determined plasma levels of H and XO from the effluent blood of a canine ileal segment after two hours of of complete ischemia followed by 30 min reperfusion. XO and H levels peaked at the beginning of the reperfusion, reaching about 14 nmol/ml/min and 12 nmol/l values respectively. Pretreatment with aminoguanidine, a blocker of diamine oxidase (histaminase) resulted in significantly higher plasma levels of H during reperfusion. This elevation was not accompanied by a further increase of XO activity. No significant change could be observed in the degree of postocclusive elevation of XO activity following intraarterial administration of 0.5-1 nanomole of H during the ischemic period. Pretreatment with the mast cell stabilizer cromolyn significantly diminished the increase in plasma levels of H at the onset of the reperfusion, but this therapy was not effective in reducing the elevation of XO activity. These data suggest, that nanomolar amounts of H do not cause an increase in the XO activity during reperfusion. The H released at the onset of the reperfusion phase is not a cause, rather an effect of the elevated activity of XO.

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IMMUNOAFFINITY LOCALIZATION OF XANTHINE OXIDASE ON THE OUTSIDE SURFACE OF THE ENDOTHELIAL CELL PLASMA MEMBRANE. H. Schiller*, S. Vickers*, J. Hildreth*, I. Mather*, F. Kuhajda*, and G. Bulkley.
The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Toxic oxygen metabolites generated from xanthine oxidase (XO) at reperfusion trigger substantial post-ischemic injury in many organs. To specifically localize XO in endothelial cells (ec) at a subcellular level, we prepared both rabbit polyclonal (PAb) and murine monoclonal (MAb) antibodies to affinity-purified XO. Both produced classic Ag-Ab binding curves. The specificity of these Abs for XO was confirmed by immunoprecipitation of only XO from human liver homogenate, Ouchterloney gel immunodiffusion against XO which produced only one precipitate band, and inhibition of XO enzymatic actitivy in vitro by immunoprecipitation with the PAb, or incubation with the MAb. Immunoaffinity labeling of tissue sections localized XO in the microvascular endothelium of virtually all organs, in all species tested, including pig and man, and in cultured bovine and porcine endothelial cell (ec) monolayers. Immunofluorescent staining of viable, intact ec (impermeable to immunoglobulins) showed strong (grade 3/4 by blinded reading) and specific [blocked by XO (0/4)] staining of the outer surface of the plasma membrane. No staining was seen either with nonspecific IgG (0/4), nor with a MAb against the exclusively intracellular protein, actin (0/4). Only when ec were permeabilized did their cytoplasm stain strongly for both actin (4/4) and XO (4/4). In separate experiments, intact ec bound strongly to anti-XO fixed to petri dishes (116±7 cells/hpf) [Viability (impermeability) of these ec was confirmed by their subsequent growth and replication.] Binding was blocked with XO (26±7/hpf), and was not seen with nonspecific IgG (12.5±0.2/hpf). Anti-XO hybridoma cells themselves bound to the outside surface of ec plasma membrane (28±6/hpf), binding blocked by XO (3.5±0.8/hpf), and not seen with nonspecific myeloma cells (0.5±0.3 /hpf). These findings clearly indicate that XO is not only a cytoplasmic enzyme, but that it is also localized on the outside surface of the endothelial cell plasma membrane, where it is strategically located to initiate reperfusion injury and is accessible to circulating antioxidants.

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IMMUNOAFFINITY LOCALIZATION OF THE FREE RADICAL-GENERATING ENZYME, XANTHINE OXIDASE IN THE MICROVASCULAR ENDOTHELIUM OF THE PORCINE AND HUMAN BRAIN. M. Miyachi*, S. Vickers*, H. Schiller*, P. Patel*, I. Mather*, J. Hildreth*, F. Kuhajda*, and G. Bulkley.
The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Toxic oxygen metabolites generated from xanthine oxidase (XO) at reperfusion trigger substantial post-ischemic injury in several animal models of cerebral ischemia. However, XO has been documented in neither porcine nor human brain. To so localize XO by immunoaffinity, we prepared highly specific rabbit polyclonal (PAb) and murine monoclonal (MAb) antibodies to affinity-purified XO. Specificity of these Abs to XO was confirmed by antigen-Ab titration binding, the immunoprecipitation of only XO (confirmed by gel electrophoresis) from human liver homogenate, Ouchterloney gel immunodiffusion against XO which produced only a single precipitate band, and the fact that immunoprecipiation with the PAb, or incubation with the MAb, each inhibited XO enzymatic activity in vitro. Immunoperoxidase staining of fresh frozen and glutaraldehyde-fixed sections of porcine and human temporal lobe cortex revealed strong staining, localized only in the microvascular endothelium. The specificity of this staining for XO was confirmed by staining blockade with excess XO. No such staining was seen with a nonspecific IgG. A separate histochemical stain for XO enzymatic activity was similarly positive in only the microvascular endothelium of rat brain, and was completely blocked by allopurinol. These findings consititute the first demonstration of XO in the human (and porcine) brain, and indicate that the enzyme is localized in the microvascular endothelium where it can trigger a free radical-mediated "no reflow" phenomenon.

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REPERFUSION MUCOSAL DAMAGE FOLLOWING COMPLETE INTESTINAL ISCHEMIA IN THE DOG. **M.Boros*, G.Karácsony* and S.Nagy.**
Szent-Györgyi Albert Medical University, H-6701 Szeged, Hungary.

Conflicting data have been reported on the significance of the reperfusion period in the pathology of intestinal ischemia of different etiologies. Reperfusion mucosal damage was demonstrated following incomplete ischemia, but no substantial exacerbation of tissue injury could be detected following complete ischemia. Recently, however, reperfusion injury was demonstrated in a rat model of total ischemia, if venous congestion was avoided. Our aim was to examine this possibility in a canine model of complete intestinal ischemia, and to investigate the effect of antioxidant treatment: allopurinol (A) alone or in combination with the superoxide radical scavenger MTDQ-DA and steroid therapy: Dexamethasone (Dex) or Methylprednisolone (Mp) on postocclusive histological changes. 120 min of total arterial ischemia of an ileal segment was followed by a 30 min reperfusion period. Tissue samples taken at the end of the ischemic interval or in the 30th min of the reperfusion were evaluated histologically in a blinded manner, using the 0 - V grade scale of Chiu et al. (Arch Surg 101, 478-483,1970). Severe mucosal damage was seen in ischemia alone and in ischemia-reperfusion, too. There was no significant difference in mucosal thickness between any of the groups. Intravenous A, MTDQ-DA alone, as well as Dex or Mp were not effective in diminishing the postocclusive mucosal injury. Two days of pretreatment with oral A alone, or in combination with MTDQ-DA resulted in a significant amelioration of postischemic histological changes. Our results suggest that reperfusion injury may result even after complete intestinal ischemia and this damage can be attenuated by antioxidant therapy.

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HISTAMINE RELEASE AND SOD, ALLOPURINOL AND RANITIDINE PRETREATMENT IN HAEMORRHAGIC SHOCK IN THE RAT.

I. Zöllei, H. Asakawa, S. Karácsonyi /Spon: S. Nagy/ Med. Univ. Szeged 6720 Szeged, Hungary; Univ. Asahikawa, Japan

There are some observation that oxygen free radicals can cause histamine release. Histamine is a strong stimulus of gastric acid release. The goal of this study was to determine whether ranitidine or antioxidant pretreatment modify the release of histamine during the haemorrhagic shock. In the anesthetized rat 0.1 N HCL was instilled into the stomach and the rat was bled to reduce the blood pressure to 30 mmHg for 20 min. The shed blood was reinfused. Twenty minutes later the stomach was removed. The area of gastric mucosal lesions were measured, histological grading was made. Blood samples taken from the carotid artery were examined by radio-immuno-assay /IMMUNOTECH S. A./ to determine the plasma histamine level. The histamine level did not change significantly during the preparative surgery, but there was a significant increase of histamine level by the end of the shock period. After the reinfusion of the blood the plasma histamine remained essentially at the same for five min, and later it decreased dramatically. Allopurinol and SOD and ranitidine pretreatment significantly protected against the gastric mucosal lesions. Allopurinol and SOD did not influence significantly the values of plasma histamine level. Ranitidine caused significant histamine release immediately after the injection and the plasma histamine values were significantly higher in this group compared to the control histamine level except for the final value, which was lower than the control value.

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PLASMA LYSOZYME LEVELS IN MURINE SEPSIS - EFFECT OF ANTI-OXIDATIVE/ANTI-INFLAMMATORY THERAPY.

D. Marinkovic, N.J. Haeringen and J. Oosting.
Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

In order to obtain better orientation about the influence of anti-oxidative/anti-inflammatory combination therapy (AACT) with dimethyl sulfoxide, chlorpromazine and vitamin E upon the activity of the inflammation, plasma lysozyme level (PLL) was determined 24 hrs postoperatively in the modified caecal ligation sepsis model in the mouse (CLSM).
PLL was equal in an unoperated male and female control group (1.9 ± 0.2 mg/l).
In both operated male and female groups a significant increase of PLL was found ($p < 0.01$).
PLL was significantly higher ($p < 0.05$) in the operated male group (3.4 ± 0.2 mg/l) in comparison with the operated female group (2.6 ± 0.2 mg/l). PLL in the operated with AACT treated male (2.2 ± 0.1 mg/l) and female (1.8 ± 0.1 mg/l) group was not significantly different from the values found in the unoperated male and female group.
Increase of PLL only in operated untreated groups and not in operated groups treated with AACT suggests suppression of the activity of the inflammation by this therapy.
In accordance with changes of PLL there is also higher mortality of the operated untreated male group in comparison with the operated untreated female group in this CLSM.
The AACT applied in this CLSM reduces mortality in both male and female groups.

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ROLE OF INTRAVENOUS SUPEROXIDE DISMUTASE IN THE PROTECTION OF VASCULAR ENDOTHELIAL FUNCTION IN RAT ENDOTOXIC SHOCK

Martin R. Siegfried, Xin-liang Ma, Joseph Erhardt and Allan M. Lefer, Department of Physiology, Jefferson Medical College, Philadelphia, PA 19107

Intravenous administration of endotoxin (i.e., lipopolysaccharide) produces a well known form of circulatory shock, characterized by hypotension, decreased cardiac performance, and eventual death. The circulating lipopolysaccharide is thought to interact with the vascular endothelium and the blood cells, resulting in the elaboration of toxic mediators including the cytokines, tumor necrosis factor (TNF) and interleukin-1 (IL1), as well as oxygen-derived free radicals. We investigated whether endothelial dysfunction occurred during the course of endotoxic shock, and if intravenous superoxide dismutase (hSOD) was protective in a four hour model of endotoxin shock in rats. In this model, 30 mg/kg of E. coli. endotoxin administration resulted in a significant decrease in mean arterial blood pressure over the entire four hours and a significant transient tachycardia. Furthermore, endotoxemia resulted in significant increases in several indices of circulatory shock, notably plasma amino-nitrogen concentrations and plasma cathepsin D and myocardial depressant factor activities, intestinal myeloperoxidase activity, and hematocrit. hSOD treatment prevented the initial tachycardia and partially restored arterial blood pressure. Additionally, superior mesenteric artery (SMA) rings were harvested following endotoxin shock and their vascular responsiveness was evaluated *in vitro*. Endotoxin administration failed to decrease SMA vasoconstrictor activity to 9,11-methanoepoxy PGH_2, but endotoxemia significantly blunted vasodilator responsiveness in SMA to the endothelial-dependent vasodilators, acetylcholine and A23187. In contrast, responses to acidified sodium nitrite were not blunted. hSOD treatment resulted in a significant amelioration of endothelial dependent vasodilation to acetylcholine (33 ± 7% vs 71 ± 4%) and to A23187 (35 ± 7% vs 76 ± 5%) in endotoxin and endotoxin + hSOD treated rats, respectively ($p<0.01$). Thus, endothelium dependent relaxation in SMA was markedly impaired in endotoxin shock, but superoxide dismutase protected against this loss of vascular function.

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S23: Eicosanoids—Generation and Inhibition

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Eicosanoids and Exotoxin-Evoked Acute Respiratory Failure.

W. Seeger, D. Walmrath, and F. Grimminger
Department of Internal Medicine, Justus-Liebig University Giessen, FRG

Eicosanoids have since long been implicated in acute lung injury. We investigated the mechanisms of acute respiratory failure induced by bacterial exotoxins. In particular, we applied Staphylococcus aureus α-toxin, Pseudomonas aeruginosa Cytotoxin and Escherichia-coli Hemolysin in isolated, ventilated and blood-free perfused rabbit lungs. All three exotoxins evoke an acute increase in pulmonary artery pressure, which is predominantly mediated by pulmonary thromboxane generation. The exotoxin-evoked pressor response is amplified after a preceding period of endotoxin "priming". The pulmonary hypertension is accompanied by a severe mismatch of ventilation and perfusion. Vasoconstrictive and vasodilative eicosanoids appear to be centrally involved in this disturbance of gas-exchange. In addition, protracted lung edema formation is evoked by the bacterial exotoxins. The latter is accompanied by marked generation of arachidonic acid lipoxygenase products. The enrollment of leukotrienes and HETEs in the present model of septic vascular permeability increase is under current investigation. In conlcusion, bacterial exotoxins can fully reproduce acute respiratory failure in blood-free perfused rabbit lungs, and pulmonary eicosanoid generation contributes to the sequence of pathogenetic events.

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Changes in Eicosanoid after GI Surgery

Yoshifumi KAWARADA M.D., FACS, Makoto IWATA M.D., Hajime YOKOI M.D., Takashi NOGUCHI M.D., & Ryuji MIZUMOTO M.D., FACS, FACG,
1st Dept. of Surgery, Mie University, School of Med. Tsu, Mie, Japan

Eicosanoid demonstrates body defense reaction on the one hand, but cytotoxicity on the other, thus contributing greatly to the onset of organic disorders associated with surgical invasion.

In the present study, changes in the levels of Eicosanoid associated with major surgery, particularly their relation to the onset of post-operative complications were investigated in 94 patients who had undergone surgery of the digestive organs.

[Results & Summary] PSTI AND PMNE fluctuated after major surgery but their association with prognosis was insignificant, whereas PLA_2 activity and levels of TxB_2, 6-KF, LT and IL-1B or TNF frequently changed with the onset of organ failure. Sepsis and DIC were preceded by elevation of LBT_4, which appears to be involved in the pathogenesis of early organ failures. Recently, the effect of cytokine released from immunocytes has been given attention, particularly TNF for its wide variety of biologic activities associated with the living body's defense mechanism and cellular disorders. The greater majority of patients with detectable levels of TNF had postoperative complications, demonstrating a relationship with endotoxemia.

It was also suggested that Eicosanoid is closely related to the serum endotoxin level.

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EICOSANOIDS AS MEDIATORS OF ACUTE ISCHEMIC INJURY WITH AND WITHOUT REPERFUSION

Allan M. Lefer, Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107

Thromboxane A_2 (TxA_2) appears to be an important mediator of ischemic injury. Despite its short half-life, TxA_2 contributes to the pathogenesis of cardiopulmonary diseases (e.g., sudden death, myocardial ischemia). It does so because it propagates its own formation by activating platelets and constricting blood vessels, thus activating more TxA_2 and trapping it locally within an ischemic or hypoxic region. TxA_2 concentrations in the extracellular fluid of lymph of ischemic regions may be much higher than that occurring in non-ischemic, normally perfused regions. Additionally, TxA_2 exerts four important effects which contribute to ischemic injury including (a) aggregation of platelets, (b) constriction of blood vessels, (c) induction of bronchoconstriction, and (d) enhancement of permeability of cell membranes. Specific and potent Tx receptor antagonists (TxRA) have recently become available for study. These include pinane thromboxane A_2 (PTA_2), BM-13,505 and SQ-29,548. The TxRA are useful tools in the study of the pathophysiology of Tx-dependent disease processes and have been found to be effective in a variety of ischemic disorders including myocardial ischemia and splanchnic ischemia without reperfusion. In contrast, leukotriene B_4 (LTB_4) has been shown to induce chemotaxis of neutrophils (PMNs) and play a key role in reperfusion injury following reperfusion of an ischemic vascular bed (e.g., coronary, mesenteric bed). Recently, use of LTB_4 receptor antagonists have been found to be

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effective therapeutic agents in splanchnic and myocardial ischemia with reperfusion. The beneficial effects include prevention of increased myeloperoxidase (MPO) activity in reperfused tissues, reduced necrosis of reperfused tissue, and preservation of endothelial integrity of the vessels perfusing these ischemic beds. LTB_4 receptor antagonists are both useful tools in the study of reperfusion injury as well as effective therapeutic agents.

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IBUPROFEN ATTENUATES TNF-INDUCED EICOSANOID SYNTHESIS AND IMPROVES TNF-INDUCED MORTALITY. J. Raymond Fletcher, J. M. Moore*, M. Earnest*, A. DiSimone*, P. Williams*, N. Abumerad.
University of South Alabama, Mobile, AL and Vanderbilt University, Nashville, TN 37221 36617

TNF is a cardinal mediator in sepsis. The cellular mechanisms of TNF effects are unknown. We hypothesized that a) TNF induces eicosanoid synthesis and b) that a cyclooxygenase inhibitor would improve the survival in TNF-induced mortality (150 ng/kg/IV/5 min, ~ 90 mortality at 4 hrs). METHODS: Conscious male rats with arterial and venous catheters were randomized: Group I - TNF alone (150 ng/kg/IV/5 min), n =30; Group II - Ibuprofen (30 mg/kg/IV at t = -20 and +240 min plus TNF), n=28. Eicosanoids (TxB2, 6 keto-$PGF_{1\alpha}$, PGE_2), hemodynamics, and mortality were determined over 4 hrs. RESULTS: TNF stimulated synthesis of TxB_2 (71±30 pg/ml, mean±SEM at baseline vs 117±18 at +240 min, $p<0.02$), 6-keto-$PGF_{1\alpha}$ (54±15 pg/ml at baseline vs 250±80 at +240 min, $p<0.02$), PGE_2 (70±6 pg/ml at baseline vs 231±68 at +240 min, $p<0.02$) n=4 for each group. Ibuprofen significantly ($p<0.05$) attenuated TNF-induced eicosanoid release. TNF-induced mortality (87%, 26/30) was dramatically decreased with Ibuprofen (11%, 3/28) at 4 hrs ($p<0.001$). Hemodynamic events were similar in both groups.
CONCLUSIONS: 1) TNF induces cyclooxygenase synthesis. 2) Ibuprofen attenuates TNF-induced eicosanoid synthesis and reverses TNF induced mortality. 3) TNF effects on endothelial cells (6-keto-$PGF^{1\alpha}$), and macrophages (PGE_2), and platelets (TxB_2) are different. 4) TNF effects on hemodynamic events are not mediated via the eicosanoids. 5) Cellular effects of TNF on mortality, like those of endotoxemia/sepsis, may be mediated in part via the eicosanoids. 6) We are unaware of similar data in the literature.

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ROLE AND MODIFICATION OF EICOSANOIDS IN HUMAN SEPSIS SYNDROME. G.R. Bernard, B.B. Swindell, S. Higgins, D. Reines, C.A. Metz. Center of Lung Research, Vanderbilt University, Nashville, TN, USA.

Arachidonic acid metabolites, especially thromboxane-A2 and prostacyclin, have been shown to be increased in experimental models of sepsis and the adult respiratory distress syndrome (ARDS) and play a major pathophysiologic role. This study was designed to determine if these metabolites are increased in human sepsis syndrome and if inhibition of fatty acid cyclooxygenase affects their formation and their pathophysiologic sequelae. We conducted a double-blind, placebo controlled trial of ibuprofen (800 mg rectal every 4 hours for 3 doses) in 30 patients with sepsis syndrome defined by abnormal vital signs, the appearance of serious infection and at least one major organ failure. Urinary concentrations of the metabolite of thromboxane-A2,2,3-dinor-TxB2, and prostacyclin,2,3-dinor-6-keto-PGF2α, were elevated 10-20 times normal and declined to 4-5 times normal by 12 hours after entry in the ibuprofen treated group and remained elevated in the placebo treated patients. The urinary concentration of TxB2 and 6-keto-PGF1α which reflect renal production of TxA2 and prostacyclin, respectively, were also increased approximately 10-fold over normal and were subsequently decreased by ibuprofen. Coincident with the reduction in metabolite levels, the ibuprofen treated group, but not the placebo treated group, experienced a significant decline in temperature, heart rate, peak airway pressure, and a trend towards more rapid reversal of shock ($p=.12$) and ARDS ($p=.03$).
We conclude that 1) prostacyclin and thromboxane synthesis is markedly increased in human sepsis, 2) ibuprofen administration is relatively safe and significantly reduces arachidonic acid metabolism and the associated pathophysiology in this population, and 3) a larger trial is now warranted to determine whether ibuprofen can prevent or reverse septic shock and ARDS and improve survival.

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EFFECTS OF PGE1 ON NEUTROPHIL FUNCTIONS - IN VITRO STUDIES AND RESULTS OF A CLINICAL TRIAL WITH PGE1-TREATED POLYTRAUMA PATIENTS AT RISK OF THE ARDS. A. Dwenger, M. Nerlich#, H.C. Pape#, E. Jonas, J. Köhl§, A. Seekamp#, and G. Schweitzer (Spon: H. Redl).
Medizinische Hochschule Hannover, Abteilung für Klinische Biochemie, Unfallchirurgische Klinik# und Abteilung für Medizinische Mikrobiologie§

The aim of the study was to realize the inhibitory effects of PGE1 on neutrophil(PMNL) functions in vitro and to demonstrate the beneficial effect of PGE1 in patients at risk of the ARDS. 1) Oxygen radical production (chemiluminescence, CL), adherence and elastase (Ela) release of stimulated human PMNL were studied. 2) PMNL-induced damage of cultered human endothelial cell monolayers (EC) was followed by CL and 111-In release of labeled EC. 3) In 19 multiply traumatized patients at risk of the ARDS (9 patients with 20 ng PGE1/min·kg for 6 days; 10 placebo patients) biochemical, hemodynamic and cardio-respiratory parameters were monitored for 10 days and compared by Mann-Whitney's U-test. The results were: 1) a significant dose-dependent inhibition by PGE1 of CL response, adherence and Ela release in isolated PMNL, 2) a significant dose-dependent reduction of CL re-

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sponse and EC damage by PGE1 during EC/PMNL interaction, and 3) an observation of the 10 day-course differences between PGE1 (+) and placebo (-) patients: plasma Ela (+<-; p< 0.005), CL response of PMNL by zymosan stimulation (+>-; p<0.01) and by EC interaction (+>-; p<0.05), PMNL myeloperoxidase content (+>-; p<0.009), plasma C3a (+<-; p<0.0001). Pulmonary function and ARDS incidence were improved for PGE1 patients but not being significantly different from placebo patients. In conclusion, significant inhibitory effects of PGE1 on neutrophil functions could be substantiated in vitro. By an early PGE1 infusion in patients at risk of the ARDS the inhibition of neutrophil functions and neutrophil-mediated pathogenetic pathways was seen. It remains unanswered whether the observed amelioration of pulmonary function and ARDS incidence will become significantly different by the investigation of further patients at risk of the ARDS.

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NUTRIENT AND NON-NUTRIENT RENAL BLOOD FLOW

J.C. Passmore, J.S. Young, D.A. Hartupee, and A.E. Jimenez
University of Louisville, Louisville, Kentucky 40292

The ^{133}Xe washout, freeze-dissection technique demonstrated that blood flow values of 3.7, 2.6 and 1.5 ml/g·min exist for outer cortex, inner cortex and outer medulla, respectively. The sum of these three compartments constitutes nutrient renal blood flow (NRBF; Circ. Res. 13:290-307, 1963). Total renal blood flow (TRBF) measured electromagnetically minus NRBF revealed non-nutrient renal blood flow (NNRBF) to be 82% of TRBF. NNRBF was also measured by the injection of ^{86}Rb into the renal artery followed by quantification of non-equilibrated ^{86}Rb in the renal venous outflow. Reduction of TRBF by renal artery occlusion reduced blood flows of the outer cortex, inner cortex and outer medulla to 1.9, 1.4 and 0.8 ml/g·min, respectively, based on the ^{133}Xe technique. Control NNRBF (TRBF-NRBF) was 18%. NNRBF was unchanged at 21% during restricted flow conditions and was also 18.7% with the ^{86}Rb technique.

Ibuprofen (iv) did not alter NRBF but reduced TRBF by 13%. ^{86}Rb studies supported a selective decrease in NNRBF after ibuprofen (J. Lab. Clin. Med. 115:680-7, 1990). Acetylcholine infusion increased TRBF by 52% with no change in NRBF, thus indicating an increase in NNRBF which was verified by ^{86}Rb washout findings. With TRBF held constant by renal artery constriction during acetylcholine infusion, NNRBF was still increased indicating that the NNRBF determined by ^{86}Rb extraction was not flow dependent. We conclude that there exists a significant non-nutrient component in the renal circulation and that it may be influenced by the hormonal environment. (Supported by Eli Lilly Corp. and American Heart Assoc./Kentucky Affiliate.)

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AN ATTEMPT TO CORRELATE THE VASOACTIVITY OF A PROSTACYCLIN ANALOGUE, TAPROSTENE, WITH ITS EFFECTS ON METABOLIC ACIDOSIS IN ENDOTOXIN SHOCKED RATS.

J. Schneider
Grünenthal GmbH, Dept. of Pharmacology, D-5100 Aachen, Zieglerstr. 6

Improvement of tissue perfusion and increase of oxygen supply in septic patients has been achieved by the vasodilatating and platelet aggregation inhibiting prostacyclin. Nevertheless, because of its vasoactivity prostacyclin or its analogues are suspected to deteriorate blood oxygenation due to pulmonary AV-shunt and to further aggravate tissue hypoxia due to decreased perfusion pressure. In the present study the relationship between prostacyclin-like vasodilatation and tissue oxygenation has been investigated in endotoxin shocked rats. A prostacyclin analogue, taprostene, has been infused in the range from 0.1 - 1.0 µg/kg·min which comprises threshold and clear hypotensive doses in anesthetized rats. Infusion of E. coli LPS (25 mg/kg i.v. over 4 hrs) produced tissue hypoxia with metabolic acidosis as evident from decreased hydrogen bicarbonate (HCO_3^-) and standard base excess (SBE) levels. Infusion with 0.1 µg/kg·min taprostene significantly inhibited this metabolic acidosis. With higher doses of taprostene, that further lowered the mean arterial blood pressure (MABP) in endotoxemic rats, this protective effect gradually disappeared. MABP at the end of endotoxin infusion in taprostene-treated rats correlated with the levels of HCO_3^- ($r = 0.703$, $p < 0.001$) and SBE ($r = 0.714$, $p < 0.001$). Even the highest infusion dose of taprostene did not influence the arterial pO_2. It is concluded that the prostacyclin analogue taprostene in doses with threshold vasoactivity improves tissue oxygenation and thereby inhibits metabolic acidosis in endotoxin shocked rats; this effect fades away with higher and clear hypotensive doses, most probably due to a decreased perfusion pressure. There was no evidence for an impaired arterial oxygenation with infusion of the prostacyclin analogue.

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FAVOURABLE EFFECT OF DEFIBROTIDE IN SEPTIC SHOCK IN PIGS.

Th. Hohlfeld, G. Nowak, E. Bucha, E. Brüggener, H. Strobach, K. Schrör. (Spon: A.M. Lefer)
Institut für Pharmakologie, Heinrich-Heine-Universität Düsseldorf, W-4000 Düsseldorf and Institut für Pharmakologie und Toxikologie, Medizinische Akademie Erfurt, O-5010 Erfurt, FRG

Defibrotide (DEF) is known to stimulate the endogenous formation of

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prostacyclin, resulting in cardioprotective and antiatherosclerotic effects. This study investigates the potential of DEF to protect from endotoxic shock. Anaesthetized pigs (8 - 13 kg) were subjected to lipid A infusion (1.5 mg/kg·h). DEF (32 mg/kg·h) or vehicle (VEH) were infused i.v., starting 60 min before onset of lipid A infusion. 5/7 VEH-treated pigs died within the experimental period of 240 min, whereas 6/7 DEF-treated animals survived ($p<0.05$). DEF reduced a shock-associated increase of pulmonary arterial pressure from +213 ± 41 % (VEH) to +38 ± 16 % (DEF) above control ($p<0.05$). Shock-induced depletion of circulating platelets was diminished by DEF at the end of the experimental period (VEH: -356 ± 50, DEF: -216 ± 34 $\cdot 10^3$ platelets/µl, $p<0.05$). Collagen (2 µg/ml)-stimulated, ex vivo measured platelet ATP-secretion was decreased at the end of the experimental period in VEH-, but not in DEF-treated animals (VEH: 54 ± 12, DEF: 121 ± 20 % of control, $p<0.05$). Iliac artery segments obtained at the end of the experiments from DEF-treated pigs released markedly more prostacyclin when incubated with 1 µM bradykinin than iliac artery segments of VEH-treated pigs (VEH: 21 ± 9, DEF: 51 ± 9 nM/g·10 min, $p<0.05$). These data suggest that DEF exerts beneficial effects in lipid A-induced shock. This action may be related to a reduction of platelet activation and an enhancement of the endogenous formation of prostacyclin.

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ON THE IMPORTANCE OF LIPOXYGENASE PATHWAY IN THE PATHOGENESIS OF ENDOTOXIN SHOCK. D.W.Scheuch, W.Rudolph and W.Schwab
Medical Academy Dresden,Inst.Pathol.Biochem.O-8019Dresden,F.R.G.

To test the role of metabolites of lipoxygenase (LOX) pathway in the pathogenesis of circulatory shock we employed a selective LOX-inhibitor in a well defined experimental endotoxin shock model. (1) Alveolar macrophages (MØ) derived by bronchiolar lavage were incubated with or without calcium ionophor A 23187 for 20 min at 37°C. The LOX-metabolites were analyzed by isocratic HPLC seperation. Eluting compounds were detected by UV-absorbance at 235 nm or 280 nm.

In sham shock an increase of leucotriene B4 (LTB4) and 5-HETE could be shown only after stimulation with A 23187. 6 hours after the application of endotoxin (15mg/kg b.w., i.p.) alveolar MØ showed spontanously a significant increase of LTB4 and 5-HETE. Stimulation with A 23187 enhanced the elevation 2 to 3 times above the level of stimulated MØ of sham shock.

By pretreatment (2 hours before endotoxin application) with a selective LOX-inhibitor LXU (61mg/kg b.w.) the increase of the LOX-metabolites could be prevented. Concomitantly the mortality rate in our shock model was reduced from 65 per cent to 8 per cent.

In summary, the data suggest an important role of metabolites of the lipoxygenase pathway in the pathogeneses of endotoxin shock. The determination of LOX-metabolites could be useful for the evaluation of the prognosis of circulatory shock.

1. U.Schaper, B.Fiedler und D.W.Scheuch (1985) Z.med.lab.diagn. 26, 456

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EFFECT OF PLATELET ACTIVATING FACTOR AND LEUKOTRIENE ANTAGONISTS ON ENODOTOXIN SHOCK: ROLE OF NEUTROCYTE.
F. Goto, D. Yoshikawa, K. Uehara, and T. Fujita.
Gunma University School of Medicine, Maebashi, Gunma, Japan 371.

Platelet activating factor (PAF) has been suggested as an important mediator in endotoxin shock. We have sudied the effects of PAF antagonist CV-3988 and leukotriene (LT) antagonist ONO-1078 on E. coli lipopolisaccharide (LPS)-induced sequele in rats. Pretreatment with CV-3988 (6 mg/kg, i.v.) or ONO-1078 (150 mg/kg, p.o.) did not improve survival rates following the administraiton of LPS compared to that of control rats pretreated with solvents of the drugs. Rats pretreated with CV-3988 combined with ONO-1078 exhibited significantly enhanced survival, and the treatment inhibited change of plasma transaminase levels after LPS administration. Neutropenia induced by vinblastin increased survival rates following the administration of LPS without the pretreatment of PAF and LT antagonists. But anti-shock action of the pretreatment of CV-3988 combined with ONO-1078 was not seen in neutropenia rats. These data suggested that synergistic action of PAF and LTs is important in the pathogenesis of neutrophil activated by endotoxin shock, and they may be key mediators of neutrophil activated by LPS.

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31P-MRS STUDY OF THE PROTECTIVE EFFECTS OF PROSTAGLANDIN OLIGOMERS DURING FOREBRAIN ISCHEMIA-REPERFUSION IN RATS. M. Kurata, M. Okuda, M. Muneyuki and S. T. Ohnishi,
Department of Anesthesiology, Mie University School of Medicine, Tsu, Mie 514, Japan and PhiladelPhia Biomedical Research Institute, 502 King of Prussia Road, Radnor, PA 19087, USA.

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Two oligomeric ester-type prostaglandin compounds were synthesized, one from prostaglandin E1(termed MR-356) and the other from prostaglandin B2(termed OC-5186). The latter was found to have ten times greater antioxidative activity than the former in a mitochondrial lipid peroxidation assay. These compounds have been shown to protect organs against ischemic injury. Using *in vivo* ^{31}P-MRS, we studied the potential protective effects of these oligomers on the high energy phosphate metabolism and the intracellular pH(pHi) in the rat forebrain during ischemia-reperfusion. Forebrain ischemia was produced for 15 min by bleeding to 30-40mmHg combined with bilateral carotid arteries occlusion. Ischemia followed by reperfusion caused decreases of intracellular high energy phosphates and pHi in both the control and drug-treated groups The OC-5186-treated group recovered on reperfusion to the preischemia level more rapidly than the control group. MR-356 had more limited effects. Our results suggest that antioxidative activity of these compounds may be an important factor in their ability to protect the brain againsts ischemic damage.

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UNCLAMPING OF THE ABDOMINAL AORTA DURING SURGICAL REPAIR : EXPERIENCES WITH PROSTAGLANDIN E1. A.Gabriel, A.Griesmacher, G.Kretschmer, S.Schwarz F.X.Lackner. Vienna Sch.Med., A-1o9o Vienna, Austria.

INTRODUCTION: Reperfusion of the ischemic limbs after declamping of the abdominal aorta causes pulmonary microvascular injury due to the synthesis of Thromboxane and Neutrophil activation.The proven cytoprotective potential of Eicosanoids was the reason for its intraoperative use during aneurysmectomy of the abdominal aorta.METHODS: 14 patients have been studied, 8 being treated with Prostaglandin E1 (PGE1) vs 6 controls. In all patients of the PGE1-group a low dose of at least 3ong/kg/min was administered as basic treatment.RESULTS: Mean Thromboxane levels increased from 135 to 347pg/ml after induction of anesthesia in both groups.During aortic cross clamping the levels fell (26opg/ml) and returned to preclamp-levels during the following hours.During early reperfusion there was a 5-fold increase of Prostacyclin in the PGE1-treated group vs baseline(from 221 to 11o9), 4 hours later the level had returned to 46opg/ml.The controls maintained levels between 4oo and 7oopg/ml.Blood lactate levels in the PGE1-treated patients were higher and increased significantly in the first hour of reperfusion. Intermittent leukopenia never occured in either group, instead white blood cells increased continuously.The platelets decreased to a nadir on postoperative day2.CONCLUSION: There is no evidence, that PGE1 exerts cytoprotective actions on the ischemic tissue of the lower limbs. During reperfusion blood lactate- and Prostacyclin- levels of PGE1-treated patients are even higher. Thromboxane B2 levels were *not* affected by PGE1-treatment.

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PREVENTION OF BACTERIAL TRANSLOCATION WITH PROSTAGLANDIN E ANALOGS. L Gianotti, T Pyles, JW Alexander, M Carey. University of Cincinnati, School of Medicine; Cincinnati, OH 45267-0558.

During trauma, bacteria translocated (BT) from the bowel, could be a cause, of sepsis and consequent multiple organ failure. Prostaglandins (PG) of the E serial demonstrate immunosuppressive effects in vitro but are protective of the gut mucosa in vivo. To further investigate their role in sepsis, we studied two PGE1 analogs, Misoprostol (M) and Enisoprost (E) for their effect on bacterial translocation, following burn injury, where both immunosuppression and disruption of gut barrier function have been observed. Balb/c mice were treated with E (n=36) or M (n=36) for 3 days with two different doses (4.4 or 0.44 μg/kg day) prior a 30% full thickness burn (B) and simultaneous gavage (G) with 1 x 10^{10} ^{14}C E. coli. Control mice (C) (n=15) received B+G. Animals were sacrificed at 1,4 and 24 hours postburn. Blood, peritoneal fluid, liver, lungs, spleen and mesenteric lymph nodes were harvested aseptically. Quantitative bacterial colony counts\gm (expressing the number of viable BT in the organs) and radioactivity (dpm) (reflecting total number of BT) were performed. The higher dose of M, but no the lower, had a protective effect on the gut barrier since, in all organs, dpm was 50-fold less the C (4651271 vs 98125; $p < 0.05$). Bacterial colony counts, in the high M group, decreased according to dpm reduction. Both high and the low doses of E did not decrease translocation rate. At 24 hours, judging from the number of viable bacteria, low doses of E and M were able to enhance the organ's ability to kill translocated microorganisms by 10 and 160-fold respectively [14155(C) vs 1557 (E) and 14155(C) vs 88(M); $p<0.05$]. In conclusion, M more than E exhibits protective effects on the intestinal barrier function and both may be immunomodulatory in low doses.

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ALTERATIONS OF MYOCARDIAL EICOSANOIDS DURING REPERFUSION. E.Röth, B.Török*, S.Nagy Department of Experimental Surgery Medical University of Pécs, H-7643 Hungary.

Shifts of arachidonic acid metabolism during heart ischemia and reperfusion cause alterations of prostacyclin and thromboxane levels in heart tissue together with serious arrhythmic disorders. The aim of present experiments was to clarify whether or not the commonly used cardioprotective drugs exert effect on this metabolic cascade. In mongrel

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dogs LAD ligature was carried out for 45 min (Group I) and for 90 min (Group II) followed by one hour reperfusion. During experiments the animals received Verapamil (Ca^{2+}-antagonist), Brevibloc^R (ß-blocker) and saline (control) infusion. 6-keto PGF_1 and TXB_2 were determined by radioimmunoassay technique in both the collected venous blood and heart tissue homogenates. In Group I the concentration of prostacyclin (average value 3-6 pmol/ml) and thromboxane (8-12 pmol/ml) did not change during the coronary ligature. However, significant increase (approximately twofold) was observable in prostacyclin concentration after few minutes of reperfusion. Thromboxane release was diminished by Verapamil treatment (7,5±2 pmol/ml) comparing to values of control animals (16±3,8 pmol/ml). In Group II, increased level of PGF_1 and TXB_2 was present even at the time of LAD ligature in control animals, which additionally elevated during reperfusion (8±1,8 pmol/ml, 21±4,5 pmol/ml respectively). Characteristic effect of ß-blocker was found in Group II with depletion of prostacyclin (3,5±0,8 pmol/ml) and thromboxane (14±2 pmol/ml) at the beginning of reperfusion. The heart tissue samples from reperfused areas also showed increase of eicosanoids with significant elevation of TXB_2 in controls (in Group I: 115±9 pmol/g, in Group II: 142±8 pmol/g). Verapamil and Brevibloc^R could diminish not only the quantities of prostacyclin and thromboxane in both groups but also could improve the ratio of these metabolites. Our results confirm that the cardioprotection of Verapamil and Brevibloc^R could partly be explained by their effect on arachidonic acid pathway.

ENDOTOXIN STIMULATED ARACHIDONIC ACID METABOLISM; EFFECT OF PROTEIN KINASE C INHIBITION. 456

J. Geisel*, J.A. Cook, S.H. Ashton*, W.C. Wise and P.V. Halushka.
Medical University of South Carolina, Charleston, S.C. 29425, U.S.A.

The intracellular mechanisms associated with bacterial endotoxin (LPS) stimulation of macrophage (MØ) arachidonic acid (AA) metabolism have not been clearly defined. The demonstration that transcription and translation inhibitors prevent MØ AA metabolism induced by LPS or protein kinase C (pkC) activators suggest that *de novo* protein synthesis is essential. Similarly, inhibitors of pkC block LPS stimulation of MØ AA metabolism suggesting that pkC activation is an intergral part of the LPS signal transduction mechanism. These observations prompted an assessment of LPS-induced proteins in rat peritoneal MØ using ^{35}S-methionine-pulse-labeling and SDS-PAGE gel electrophoresis techniques. The effects of the transcription inhibitor, actinomycin (ACT) D, and the pkC inhibitor, staurosporine (STA), on LPS induction of proteins were determined. ACT D (10µM) or STA (0.1µM) were incubated with adherent MØ for 60 minutes prior to *Salmonella enteritidis* LPS (10µg/ml). Neither ACT D nor STA significantly altered cellular viability measured by trypan blue exclusion and lactic dehydrogenase release. ^{35}S-methionine (125µCi/ml) incubation for 60 minutes followed 60 minutes of LPS stimulation. Total protein was then collected for SDS-PAGE gel electrophoresis. SDS-PAGE demonstrated LPS stimulated increased ^{35}S-methionine incorporation at six band locations not observed in unstimulated cells. These were at molecular weights of 126kD, 77kD, 61.5kD, 43.5kD, 39kD, and 32kD. Inhibition of protein synthesis with ACT D eliminated all LPS stimulated ^{35}S-methionine incorporation. Inhibition of pkC by STA demonstrated selective inhibition of ^{35}S-methionine incorporation in the 31kD and 126kD bands. Since inhibitors of protein synthesis block AA metabolism induced by pkC activators, these data suggest that 1) pkC activation by LPS is linked to *de novo* synthesis of specific proteins and 2) the latter are essential for LPS induced MØ AA metabolism. Supported by NIH GM27673.

LEUKOTOXIN,9,10-EPOXY-12-OCTADECENOATE: A POSSIBLE RESPONSIBLE FACTOR FOR CIRCULATORY SHOCK AND DISSEMINATED INTRAVASCULAR COAGULATION. 457

M.Hayakawa,Y.Hanaki,H.Kamiya,M.Ohno,S.Sugiyama, and T.Ozawa

Department of Biomedical Chemistry,Faculty of Medicine,University of Nagoya, Tsuruma-Cho 65,Showa-ku,Nagoya,466 Japan

Purpose: We have demonstrated that neutrophils biosynthesized linoleate epoxide,9,10-epoxy-12-octadecenoate,from linoleate. This epoxide shows a highly cytotoxic effect,and is named leukotoxin. The aim of this study is to elucidate whether or not leukotoxin exits in plasma from patients with circulatory shock and disseminated intravascular coagulation(DIC) **Methods:** A series of heparinized blood samples from two patients were collected,and leukotoxin was extracted.Blood samples from normal volunteers for the control were also analyzed. **Results:** We detected leukotoxin in plasma from two patients with infectious endocarditis and circulatory shock. Maximal leukotoxin levels were 580 µM and 880 µM,respectively. Leukotoxin level were affected by the treatment.DIC was confirmed by blood coagulation studies in these two patients. In contrast,leukotoxin was not detected in plasma of normal volunteers. **Conclusion**: Leukotoxin synthesized by recruited neutrophils might be a contributory factor to circulatory shock.

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458 GRANULOCYTE ACTIVATION AND COMPARISON WITH PLASMA LEVELS OF VARIOUS MEDIATOR SYSTEMS IN PROLONGED ENDOTOXAEMIA K. Hörstmann-Jungemann, B. Klosterhalfen, E. Manegold, S. Bender, P. Vogel and C.J. Kirkpatrick Dept. of Pathology, Biochemistry and Surgery, The Technical University of Aachen, 5100 Aachen, FRG

In an animal model (domestic pigs 28-32 kg) endotoxaemia was induced by intravenous application of an E. coli endotoxin (WO111:B4). In this experiment (continued for a maximum of 18 h or until death) the animals received antibiotics and 3x LPS (0.5 µg/kg, infusion time: 30 minutes) after 0, 5, and 10 h. The plasma levels of selected mediators were measured after each LPS application. Polymorphonuclear granulocytes were isolated from (4 ml blood) at various times after the zero point 0 (immediately before the first LPS infusion). These intervals were 30,45,60,300,330,345 and 360 min, the second LPS infusion being at 300 min. The isolated PMNs were activated with A 23187 and the LTB_4-release was measured with HPLC. The eicosanoid's, $TNF_{1\alpha}$ and PAF were measured with RIA, whereas IL-6 was measured by bioassay.

The release of LTB_4 and the corresponding plasma values were compared with time of endotoxin application. After LPS application the number of isolated PMNs decreased, whereas the measured LTB_4 value (calculated to 10^7 PMNs/ml) increased. These changes in granulocyte activation correspond temporally with the LPS-induced alterations in the plasma levels of the other mediator systems tested, including $TNF_{1\alpha}$ and the cyclooxygenase products of arachidonic acid (TxB_2, PGI_2).

459 MONOCYTE THROMBOXANE A_2 RECEPTORS: A UNIQUE RECEPTOR SUBTYPE.
T. Robin Simmons*, James A. Cook, Perry V. Halushka and James N. Moore.
Medical Universitys of South Carolina, Charleston, SC 29425, College of Veterinary Medicine, Univ. of Georgia, Athens, GA, USA.

Thromboxane (TX) A_2 is a pathogenic mediator of endotoxic shock and monocytes are target cells in endotoxemia. This study tested the hypothesis that monocytes possess TXA_2 receptors which modulate monocyte function. Radioligand binding studies were performed on membranes prepared from equine peripheral blood monocytes using ^{125}I-BOP, a TXA_2 receptor agonist. ^{125}I-BOP bound to a single class of binding sites (K_d of 1.0±0.3nM and a B_{max} of 383±180 fmoles/mg protein; n=5). ^{125}I-BOP binding was saturable and displaceable by TXA_2 receptor agonists and antagonists. I-BOP (0.25nM-1µM) inhibited ($P<0.05$) monocyte chemotactic responses to zymosan activated plasma. The effect of I-BOP on chemotaxis was inhibited ($P<0.05$) by SQ29548, a TXA_2 receptor antagonist. The potential second messenger system(s) responsible for TXA_2 receptor signal transduction were investigated. In contrast to its effects in other cell types, I-BOP failed to increase intracellular-free calcium in equine monocytes. However, I-BOP significantly increased cAMP formation in a dose-dependent fashion from 5.4±0.5 to a maximum of 68.5±11.1fmoles cAMP/µg protein ($P<0.05$; n=5) in monocytes. This latter response is also in contrast to the effect of TXA_2 agonists on cAMP formation in platelets. The TXA_2 receptor antagonists SQ29548 and L657,925 blocked I-BOP stimulated cAMP formation by 72% and 77% respectively, but did not block PGE_2 (100nM) stimulated cAMP formation. The data provide evidence for: 1) the presence of a functional TXA_2 receptor on monocytes and 2) suggest a novel second messenger system for this receptor. The results also support the hypothesis that the monocyte TXA_2 receptor may represent a unique TXA_2 receptor subtype. (Supported by NIH GM27673 and NIH HL36838).

S24: Antibody Therapy Against TNF, and Adherence

460 TNF-ANTIBODIES (CB0006) IN A SUBCHRONIC SEPTIC MODEL IN BABOONS TO PREVENT MULTI-ORGAN FAILURE (MOF). G. Schlag, H. Redl, J. Davies*. Ludwig Boltzmann Institute Exp. Clin. Traumatol., Vienna, Austria; * Roodeplaat Research Lab., Pretoria, South Africa.

The aim of our study was to prevent multiple organ damage with antibodies to human TNF in a subchronic septic baboon model under intensive care conditions and to improve the survival rate.

Methods: 14 adult male baboons (19 - 23 kg body weight) were either used as controls (5×10^8 - 1×10^9 CFU/kg body weight of live E. coli) or treated with 15 mg anti-TNF-AB (CB0006) two hours before E. coli infusion. Animals were either observed until death or sacrificed after 72 hours using a new subchronic baboon model under intensive care conditions (fluid administration, supported ventilation at certain intervals).

Results:

Protective efficacy of monoclonal antibody in a septic baboon model.

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Pretreatment (2 hours)	Challenge	Survivors/Total	% Survival
Saline	5×10^8 - 1×10^9 CFU E.coli/kg BW	2/8	(25)
CB0006 15 mg/kg BW	5×10^8 - 1×10^9 CFU E.coli/kg BW	6/6	(100)

Conclusion: Administration of TNF-AB protects baboons against subsequent challenge with live E. coli in concentrations for 5×10^8 to 1×10^9 CFU/kg. With this challenge the mortality of control animals was 75 % despite intensive care conditions.

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ANTI-TNF ANTIBODY TREATMENT OF GRAM-POSITIVE SEPSIS IN NONHUMAN PRIMATES: PROTECTION AGAINST MULTIORGAN FAILURE AND LETHALITY L.B. Hinshaw, T.E. Emerson, Jr., F.B. Taylor, Jr., and M.A. Fournel*. Oklahoma Medical Research Foundation, Oklahoma City, OK 73104 and Cutter Biological, Miles Inc., Berkeley, CA 94701 (USA).

The clinical significance of Gram-positive sepsis has been grossly underestimated. The purpose of this study was to develop a nonhuman primate model of Staphylococcus aureus (S. aureus) - induced septic shock and test the efficacy of antibody to TNF-α in preventing organ pathology and death. Experiments were carried out on 12 anesthetized young adult baboons, Papio c. cynocephalus, given intravenous doses of S. aureus, 4×10^{10} CFU/kg, during a 2 hr period. Thirty minutes after the onset of S. aureus infusion, monoclonal antibody to TNF-α, 15 mg/kg, was administered intravenously. The antibiotic, ceftriaxone, 100 mg/kg, was given intravenously at 5 hrs and intramuscularly (50 mg/kg), at 24, 48 and 72 hrs. Animals were monitored for 10 hrs., observed continuously for 30 hours and daily for 7 days or until death. Five of 6 control baboons (S. aureus + antibiotic) died between 18 and 112 hrs (mean, 54 hrs.); all 6 experimental animals treated with S. aureus+antibiotic+antibody to TNF survived longer than 7 days and were euthanized on the 8th day for histological evaluation. Mean systemic arterial pressures did not change in either group for 10 hrs. Serum TNF concentrations reached an average peak concentration of 480 picograms in 2 hrs in the control group, but were unmeasurable in all antibody-treated animals. Multisystem organ failure as evidenced by histological examination, included lungs, adrenals, liver, spleen and kidneys in control animals but was absent or diminished in adrenals, liver, spleen and kidneys of the antibody-treated group. Lung pathology was not prevented by antibody treatment but adrenal and spleen pathology was essentially abolished by antibody therapy. Major findings in organs of control animals were vascular congestion, hemorrhage, presence of fibrin thrombi, neutrophil and bacterial colony accumulations and necrosis. Findings underscore the major role of TNF in the pathogenesis of S. aureus-induced shock.

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Comparison of Efficacy of Monoclonal Antibody against Human TNF-Alpha (TNF MAb) in Swine and Baboon Models of Lethal E. Coli Bacteremia. T.E.Emerson, Jr., D.C.Lindsey*, G.J.Jesmok, M.A.Fournel* and L.B.Hinshaw. Cutter Biological, Miles Inc., Berkeley, CA 94701 and Okla.Med.Res.Found., Oklahoma City, OK 73104.

We have previously reported that threatment with TNF MAb os efficacious in an LD100 E. coli bacteremic baboon model (Circ. Shock, 30:279, 1990). In the present study, we evaluated efficacy of TNF MAb in an LD100 E. coli bacteremic pig model and compared selected parameters between the respective models. Juvenile pigs were treated with TNF MAb (15 mg/kg; n=5) or its excipient (n=12) immediately prior to an i.v. bolus of E. coli bacteria (8.9 x 10e8 cfu/kg). Blood samples were taken at baseline and 2 hrs postchallenge; all pigs then recieved 5 mg/kg i.m. gentamicin, followed by 3 mg/kg i.m. daily through 72 hrs. Survival through seven days was considered permanent. Plasma TNF was determined by the WEHI cell cytotoxicity assay. Mortalitiy in control pigs was 100%, plasma TNF peak at 2 hrs (2.5 ng/ml) and WBC count decreased by 88% at 2 hrs. In the TNF MAb group, mortalitiy was 0%, no TNF was detectable and the WBC decreased by only 19%. Comparing pig vs baboon, mortalitiy was the same, peak TNF levels were 2.5 ng/ml vs 70 ng/ml, respectively, and leukopenia was the same at 2 hrs. TNF MAb treatment was equally efficacious except leukopenia was unaffected in the baboon.

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MONOCLONAL ANTIBODIES TO TUMOR NECROSIS FACTOR-α ANTAGONIZE LETHAL SEPTIC SHOCK IN ADULT RHESUS MONKEYS

V. B. Fiedler, I. Loof, E. Sander, C. Galanos*, M.A. Fournel**, (Spon: H. Redl)
Bayer AG, Pharma Research Center, D-5600 Wuppertal 1, *Max-Planck-Institute of Immunobiology, D-7800 Freiburg, both FRG, and **Cutter Biologicals, Berkeley, CA 94710, USA

Notes

In a septic shock model (established by intravenous administration of 300 mg/kg D-galactosamine + 0.1 μg/kg lipopolysaccharide [LPS] from Salmonella abortus equi) hemodynamics, blood gases, hematology, clinical chemistry, and blood plasma concentrations of tumor necrosis factor-α (TNF) were monitored for 6 hrs, and after 24 hours. At 30 min post LPS either 15 mg/kg anti-TNF monoclonal antibodies (TNF-MoABs, n=6) or vehicle-placebo (n=4) were given i.v.

During the acute 6-hrs experiment, physiological organ function was not different between the groups. However, MoABs to TNF afforded morphological protection to heart, lung, liver and kidney damage after LPS challenge. Coagulation responses (platelet count, fibrinogen, antithrombin III, thrombin-anti-thrombin III complex) were smaller in TNF-MoAB-treated monkeys. Plasma TNF levels (WEHI cytotoxicity assay) reached a peak at 60 min after LPS (350 pg/ml) in vehicle controls but no detectable TNF occurred in MoAB-treated monkeys. All control animals died 30-46 hrs after LPS with multi organ failure. All TNF-MoAB-treated animals survived 14 days ($p < 0.05$ vs. placebo group mortality).

The study indicates protection against LPS shock by TNF-MoABs which may have clinical relevance in human sepsis.

464 TNF-BINDING PROTEIN, THE SOLUBLE FORM OF THE TNF RECEPTOR p60 CHAIN AS A NATURAL INHIBITOR OF TNF ACTIVITY. A. Himmler, I. Maurer-Fogy, and G.R. Adolf (Spon: H. Redl).
Ernst Boehringer Institut, Bender & Co., Dr. Boehringergasse 5-11, A-1121 Vienna, Austria

TNF-binding protein (TNF-BP), a cysteine-rich glycoprotein of 30 kD originally purified from human urine, was shown after molecular cloning of the cDNA to represent the extracellular domain of the cell surface TNF receptor (TNF-R) p60 chain. Analysis of natural TNF-BP, cDNA and genomic sequences of the TNF-R revealed that TNF-BP is released from the cell surface by proteolytic cleavage close to the transmembrane domain of the TNF-R. Mammalian cells were stably transfected with an engineered human TNF-R cDNA and constitutively secrete soluble TNF-BP into the media. Highly purified recombinant and natural TNF-BP were compared by protein analysis and biological assays. The natural and recombinant proteins were indistinguishable in their ability to inhibit the biological (cytotoxic) activity of human TNF-α, TNF-β and murine TNF-α *in vitro*.

465 ROLE OF ENDOTHELIAL AND LEUKOCYTE ADHESION MOLECULES IN VASCULAR INJURY: THERAPEUTIC IMPLICATIONS. A.B. Malik. The Albany Medical College, Albany, NY 12208.

Several adhesion molecules on the endothelial surface (i.e. ICAM-1, ELAM-1, and GMP-140) promote neutrophil (PMN) adhesion and migration across the endothelial barrier. ICAM-1 interacts with leukocyte CD18 integrin and ELAM-1 recognizes a carbohydrate ligand on the PMN surface (sialyl Lewis X); the ligand for GMP-140 is currently unknown. We have shown that adhesion of PMN to endothelial cells mediated by either ICAM-1 or ELAM-1 is critical for the development of vascular injury. Expression of endothelial adhesion molecules causes PMN to "hyperadhere" to the endothelial cell membrane. Once the adherent PMN are activated, the released secretory products have the potential of injuring the endothelial barrier. The attached PMN are capable of delivering oxidants and proteases in a "directed" manner towards the endothelial cells to which they are adherent. In addition, expression of endothelial adhesion molecules causes activation of the adherent PMN; that is, the binding of ELAM-1 to its ligand on PMN causes PMN activation. These studies point to the critical role of endothelial cell "activation" defined as expression of ELAM-1 and ICAM-1 as the step responsible for PMN adhesion and activation. Monoclonal antibodies (mAb) directed against endothelial adhesion proteins are effective in preventing vascular injury as is the case for antibodies directed against neutrophil CD18 integrin. However, from a therapeutic point of view, mAb directed against ICAM-1 or ELAM-1 are likely to be more useful since they are targeted specifically towards endothelial cells in which adhesion molecules are expressed. Therefore, expression of adhesion molecules on the endothelial cell membrane in response to cytokines, endotoxin, and thrombin may be responsible for PMN uptake and activation in the microcirculation, and hence is a critical step in the pathogenesis of vascular injury and tissue inflammation.

466 THERAPEUTIC USES OF ANTI-ADHESION ANTIBODIES. C.L. Rice, Department of Surgery, Harborview Medical Center, University of Washington, Seattle, USA.

Neutrophils (PMN) are essential for host defense, but the possibility that the same weapons they use against pathogens could be used against the

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host was recognized over 100 years ago by Metchnikoff. A key step in PMN activity is adherence, which is mediated by glycoproteins on the PMN cell surface. An antibody to the common ß-chain of these glycoproteins, designated MAb 60.3, has been shown in vitro to block PMN-endothelial cell (EC) adherence.

In vivo experiments have shown that MAb 60.3 blocks PMN-EC adherence in a feline model of intestinal ischemia-reperfusion and in rabbit tenuissimus muscle. We tested the hypothesis that MAb 60.3 blocked organ injury and reduced mortality after hemorrhagic shock. Two sets of experiments in NZW rabbits, one involving pre-treatment, the other involving antibody administration at the time of resuscitation, demonstrated marked improvement in mortality and organ injury with antibody therapy. An additional study was performed in sub-human primates, and demonstrated marked reduction in fluid requirement over 24 hours in the antibody group, as well as the elimination of hemorrhagic gastritis.

Other studies have demonstrated prevention of tissue injury after prolonged ischemia-reperfusion and frostbite models. These data suggest that PMNs play a key role in tissue injury, and that therapy which blocks PMN-EC adherence can substantially reduce that injury.

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Possible Mechanisms of Protection afforded by TNF-Alpha Monoclonal Antibody (TNF MAb) in E. coli Challenged Pigs. G.Jesmok, D.Lindsey*, M.Fournel* and T.Emerson, Jr., Cutter Biological, Miles Inc., Berkeley, CA 94701.

Treatment with TNF MAb prevents death in LD_{100} E. coli challenged baboons and pigs. The mechanism(s) whereby TNF MAb manifests this protective effect remains to be ascertained. In order to investigate this question we monitored anaesthetized pigs (arterial and venous catheters) over a 5 hour period following acute intravenous E. coli challenge (5×10^8 cfu/kg). Mean arterial pressure (MAP) was continuously monitored and arterial blood samples were collected hourly for the measurement of leukocytes, platelets and hematocrit (Hct). Pigs were pretreated with 15 mg/kg TNF MAb (n=8) or a similar volume of excipient (n=8) followed by bolus E. coli infusion. MAP was significantly higher (1-5 hrs post E. coli) in the TNF MAb treated group, (MAP 5 hrs, excipient 56 ± 6 mmHg vs TNF MAb 85 ± 5 mmHg). The leukopenia and thrombocytopenia characteristic of the acute inflammtory cellular response to E. coli challenge was attenuated in the TNF MAb treated group (Leukocytes 5 hrs, excipient 3.0 ± 0.5 x $10^3/mm^3$ vs TNF MAb 16.1 ± 1.8 x $10^3/mm^3$, Platelets 5 hrs, excipient 280 ± 30 x $10^3/mm^3$ vs TNF MAb 418 ± 34 x $10^3/mm^3$) while the rise in hematocrit was diminished (hct 5 hrs, excipient 36.5 ± 2.3% vs TNF MAb 28.7 ± 2.1%). These results demonstrate that TNF MAb treatment maintains circulatory function in the E. coli challenged pig perhaps by attenuating neutrophil and/or platelet-endothelial interactions. The diminished rise in Hct may represent decreased microvascular leakiness in the TNF MAb treated group and may reflect lessened endothelial damage.

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BENEFICIAL EFFECTS OF ANTI-TNF MONOCLONAL ANTIBODY (mAb) IN ENDOTOXIN-INDUCED COAGULATION DISORDER IN RATS. S. Bahrami, Redl H., G. Leichtfried, C. Kober, C. Wilfing, Schlag G. Ludwig Boltzmann Institute Exp. Clin. Traumatology, Vienna, Austria

Recently, TNF has been considered to be an initial mediator for the pathophysiolgic changes following gram-negative sepsis. Therefore, we have attempted to evaluate the role of TNF in disseminated intravascular coagulation (DIC) and to determine whether mAb used against TNF alters the course of these TNF-related disorders.

Methods: We used an endotoxic shock model in rats: Experimental groups, of ten animals each, received anti-mouse TNF mAb (TN3 20 mg/kg BW) or control preparations followed by LPS (15 mg/kg i.p.) at various times (seven/two hours after or simultaneously with TN3 supply).

Results: Anti-TNF mAb significantly lowered the peak TNF levels seen two hours after LPS inoculation. Prior administration of TNF mAb mitigated the severity of the coagulation disorder induced with high LPS doses in rats.

TN3 reduced the 6-day mortality in rats dependent on the time point of the pretreatment as follows: seven hours, 100 => 60 %; two hours, 88 => 22 %; and simultaneously, 88 => 37 %.

Conclusion: Our data suggest that TNF is partly responsible for DIC and toxicity induced by high LPS doses in rats.

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ANTI-TNF IgG MONOCLONAL ANTIBODY (CB0006) IN SEPSIS SYNDROME: PHARMACOKINETICS, SAFETY AND ROLE OF PRETREATMENT IL-6 AS A PREDICTOR OF TIME OF SURVIVAL. C.J. Fisher, S.M. Opal*, J.F. Dhainaut*, J. Zimmerman*, P. Nightingale*, S.J. Harris**, R.L. Schein*, E.A. Panacek, J.L. Vincent, G.E. Foulke**, E.L. Warren*, C. Garrard*, G. Park*, M.W. Bodmer**, S. Stephens**, J. Cohen*, G. van der Linden*, J.C. Sadoff* and CB0006 Sepsis

Notes

Study Group. Center for Critical Care Research, Case Western Reserve University, 2074 Abington Road, Cleveland, Ohio 44106, USA. ** Celltech Ltd., Slough, U.K.

80 patients with sepsis syndrome were studied prospectively to determine the safety and pharmacokinetics of 4 doses (0.1mg/kg, 1.0mg/kg, 10.0mg/kg, and 1mg/kg twice) of CB0006, a murine monoclonal IgG anti-tumor necrosis factor antibody. Pretreatment IL-6, APACHE II and appropriate chemistries were obtained. Patients were followed for 28 days. CB0006 was well tolerated with no significant side effects.

	CB0006 DOSE GROUP				
PRETREATMENT VALUES	0.1mg/kg	1.0mg/kg	10mg/kg	1.0mg/kgx2	ALL GROUPS (N)
n	19	19	20	22	80
APACHE II (Median)	26.0	24.0	23.5	26.5	24.0
Log_{10} IL-6 (Mean ± SD)	3.37 ±1.05	3.12 ±1.12	3.83 ±0.86	3.16 ±0.98	3.1 ±1.0
$T_{1/2}$ (Median)	35.9	41.6	43.4	39.7	40.1

42/44 patients tested at day 14 developed significant HAMA response. The median $T_{1/2}$ for CB0006 was 40.1 hours. The peak concentration and AUC increased proportionately to the dose increase. The pharmacokinetics are best described by a one-compartment model over the range of doses studied. Pretreatment IL-6 levels (medgenix assay) strongly predicted time of survival. High pretreatment IL-6 predicted early death while low pretreatment predicted late death or survival (p=0.03). There was no difference in the 4 dose groups with respect to survival time. CB0006 appears to be safe and well tolerated and offers a potential new immunotherapeutic treatment strategy in the management of sepsis.

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Additional Abstracts

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CURRENT CLINICAL USE OF HYPEROSMOTIC/HYPERONCOTIC SOLUTIONS. G.C. Kramer. Department of Anesthesiology, University of Texas Medical Branch, Galveston, TX 77550, USA

Hyperosmotic 7.5 % saline (HS) alone or combined with a hyperoncotic colloid such as dextran (HSD) or hetastarch (HSS) has been extensively studied in animals for volume replacement. The present review focuses on both efficacy and side effects of clinical hypertonic therapy. Infusions of HSD, 250 ml, were used for prehospital resuscitation of hypovolemic trauma (4 centers, N = 294). HS and HSD, 250 ml, has been used in emergency room resuscitation (2 centers, n = 125). HSD, 4 ml/kg has been used for intraoperative volume replacement during correction of aortic aneurysm (n = 10) and HSS, 4.5 ml/kg, has been used during cardiopulmonary bypass (n = 15). The use in ICU's includes HS for volume during refractory shock (n = 12) and heart failure (n = 6); HS and HSD for post-coronary bypass grafting (n = 30); and HSS treatment of sepsis/respiratory failure (2 centers, n = 61). No significant deleterious effects were reported in the above studies, despite development of hypernatremia and hyperchloremia in all studies and a hyperchloremic acidosis in some. In prehospital resuscitation, HSD produced favorable trends in overall survival, but was only statistically significant in subpopulations (head injury & most severely injured). Improved cardiovascular function was reported in all studies. In closely monitored ICU patients, the most prominent finding was increased cardiac index, with and without increased blood pressure, reductions in peripheral resistance and increased indices of contractility. All the above published clinical trials with HS, HSD and HSS suggest safety and efficacy. However, theoretical clinical concerns remain focused on dangers of hypernatremia, hyperchloremia and their effects on dehydration and acid-base balance and a potential exacerbation of internal bleeding with surgically uncontrolled hemorrhage. The establishment of definitive indications and contraindications for HS await extensive clinical experiences.

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PRESSURE DRIVEN HEMORRHAGE: A NEW CONCEPT IN EXPERIMENTAL SHOCK. M Rocha e Silva. Research Division, The Heart Institute, São Paulo University, Caixa Postal 11450, CEP-05499, São Paulo, Brasil.

Pressure driven hemorrhage is a new concept in the experimental investigation of hypovolemic shock. It is based on the concept that arterial bleeding is linearly proportional to prevailing mean arterial pressure. Therefore, an initial bleeding rate (BR_0) is set. This reflects the dimension of the vascular lesion. Once initiated, bleeding induces hypotension, which in turn reduces the driving force for further blood loss. Therefore the rate of bleeding is continuously varied to remain proportional to mean arterial pressure (MAP). After a given interval, fluid resuscitation may be started, but blood loss may, or not be interrupted. This model mimics situations in which field resuscitation is able, or unable to interrupt blood loss. In the 2^{nd} case, the resultant increase in MAP will induce a higher rate of blood loss. It will be demonstrated that in spite of the fact that resuscitation induces higher blood loss, it is accompanied by a general improvement of hemodynamic condition. The effects of various alternative resuscitative procedures shall be described.

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PLASMA AMINO ACID AND METABOLITE INTERACTIONS IN SEPSIS. C. Chiarla*, I. Giovannini, J.H. Siegel, M. Castagneto*. MIEMSS Shock Trauma, Univ.of MD, Baltimore, MD 21201 (USA) and Shock Center CNR, Clin. Chir. Catholic Univ. (Prof. F. Crucitti), 00168 Rome, Italy

The impact of high-dose branched chain amino acid (BCAA) support on metabolic profile was assessed by performing 364 plasma-amino acidograms and dosages of plasma and urinary metabolites in 16 septic pts. Randomly, 8 pts received a 49% BCAA mixture (High-BC) and 8 pts a 16% BCAA mixture (Low-BC), with equivalent glucose, fat and total amino acid (AA) intakes. High-BC, compared to Low-BC, resulted in lower plasma μM/L of glutamine (450 vs 602), arginine (84 vs 114), proline (155 vs 372), histidine (66 vs 124), methionine (33 vs 80), asparagine (38 vs 76), serine (101 vs 122), α-amino-isobutyric acid (9 vs 24), and higher taurine (117 vs 81)($p<.01$ for all by ANOVA). These differences took place in the absence of glutamine, taurine and asparagine intake, and were opposite to those expected from differences in intake of individual AA. Differences in concentration of other AA, for High-BC vs Low-BC, were more concordant with differences in intake, but High-BC showed lower slopes in the relationships of concentration to intake ($p<.01$). High-BC vs Low-BC also had lower plasma lactate (1.3 vs 1.9 mM/L), respiratory quotient (.83 vs .87) and urinary excretion of 3-methyl-histidine (526 vs 581 μM/24 hrs), as well as higher plasma cholesterol (2.8 vs 2.2 mM/L)($p<.05$ for all). Plasma α-amino-isobutyric acid explained one third of the AA-related variability of urinary 3-methyl-histidine ($p<.01$), being directly related to it. These results, the calculation of AA clearances and regressions analysis of substrate changes in metabolic pathways of individual AA, indicated that protein catabolism in septic muscle parallels an impairment of intracellular AA transport system "A" and that there is altered hepatic synthesis and clearance of some amino acids and metabolites, from abnormal liver-muscle interactions. These abnormalities seem to be moderated by increasing availability of BCAA.

IL-8 DURING SEPTIC SHOCK, ENDOTOXEMIA, AND FOLLOWING IL-1 ADMINISTRATION

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K. J. Van Zee, L. E. DeForge, E. Fischer, M. A. Marano, J. S. Kenney, D. G. Remick, S. F. Lowry, L. L. Moldawer; Laboratory of Surgical Metabolism, Cornell University Medical Center, 525 E. 68th St., F2016, New York, NY 10021 USA (Sponsor: H. Redl)

Sepsis and endotoxemia induce a complex cascade of endogenous mediators which influence metabolic and immunologic responses in the host. Interleukin-8 (IL-8), a protein produced by a variety of cells *in vitro* in response to stimulation with LPS and proinflammatory cytokines, and known for its leukocyte activation and chemoattractant properties, is another potential mediator of host response to injury and infection. The objectives of this study were to determine whether IL-8 circulates during lethal septic shock and sublethal endotoxemia, to evaluate whether IL-1 can independently induce IL-8 production *in vivo*, and to examine the temporal relationship of IL-8 appearance with other proinflammatory cytokines and circulating leukocyte levels. Baboons (*Papio sp.*) were infused with one of the following treatments: an LD_{100} dose of live *E. coli* (10^{11}/kg), *Salmonella typhosa* LPS (500mg/kg), recombinant human IL-1α (100mg/kg), or carrier alone (0.25 mg/kg human serum albumin). Blood samples were obtained every 30-60 min during the 8-hr post-treatment observation period and cytokine levels were determined. IL-8 levels rose within 1-2 hours of administration of *E. coli*, LPS, and IL-1α and peaked at levels which correlated with the severity of the insult. Peak circulating IL-8 concentrations occurred after those of TNF and IL-1β, and simultaneously with those of IL-6. All treatment animals developed a severe neutropenia within 1-2 hours. In baboons administered a sublethal dose of LPS or IL-1α, granulocyte levels rapidly recovered while IL-8 levels were maximal and while detectable TNF and IL-1 concentrations were absent. We conclude that during septic shock, endotoxemia, and following IL-1 administration, IL-8 is widely distributed *in vivo* at concentrations similar to those of other circulating cytokines, suggesting that IL-8 exerts systemic and not solely paracrine effects. Maximal IL-8 levels, like those of IL-6, occur after TNF and IL-1β peaks, and administration of IL-1α produces an IL-8 response similar to that seen in endotoxemia, suggesting a contribution by these more proximal mediators to the production of IL-8 *in vivo*. Our findings are consistent with the hypothesis that IL-8 contributes to the dynamics of circulating neutrophils *in vivo*. It can be concluded that IL-8 participates in the complex cascade of cytokine responses to infectious and inflammatory stimuli and, as such, may play a significant role in host defense and disease.

IL-1 RECEPTOR BLOCKADE ATTENUATES THE HEMODYNAMIC AND METABOLIC CONSEQUENCES OF LETHAL E. coli SEPTIC SHOCK.

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E. Fischer, M.A. Marano, K. Van Zee, A.E. Hayes, C. Rock, A.A. Hudson, R.C. Thompson, S.F. Lowry, L.L. Moldawer (spons. H. Redl) Department of Surgery, Cornell University Medical College, New York, NY. 10021

Previous studies have emphasized the proximal role of TNFα in the pathologic sequelae of Gram negative septic shock. Although passive immunization of primates with antibodies to TNFα can prevent mortality to lethal *E. coli* bacteremia, TNFα blockade also attenuates circulating IL-1ß and IL-6. Recent studies have demonstrated that IL-1α administration to healthy baboons induces hypotension comparable to that seen in sublethal endotoxemia. Therefore, the present study was undertaken to evaluate whether an endogenous IL-1 response contributes to the hemodynamic and metabolic consequences of lethal *E. coli* septic shock.

Ten female baboons (*Papio anubis*) were anesthetized and received 10^{11} cfu/kg BW of live *E. coli*. Five of the animals receiving *E. coli* were randomized to receive simultaneously 10 mg/kg BW of an IL-1 receptor antagonist (IL-1*ra*) followed by a continuous *i.v.* infusion of 25 μg/kg BW/min of IL-1*ra* for 24 hours. Control animals received an equivalent amount of human serum albumin.

Notes

Group (*p<0.05)	#total/ #survived	MAP (mm Hg)	CO (L/min)	HR (bpm)	IL-6 (U/ml)	TNFα (ng/ml)
E.coli	5/2	-62±5	-0.4±0.2	44±5	28273±1698	41.0±20.3
E.coli+IL-1*ra*	5/5	-41±7*	-0.1±0.1*	42±7	15060±2122*	34.0±20.1

IL-1ra blockade attenuated the hemodynamic collapse and improved survival in lethal *E. coli* bacteremia. We conclude that an endogenous IL-1 response contributes to the pathological changes that lead to shock and death, and that IL-1*ra* treatment offers a novel approach to block the hemodynamic and metabolic consequences to lethal Gram negative septic shock.

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PHYSIOLOGICAL AND MORPHOLOGICAL SCORING SYSTEMS TO MONITOR ORGAN FAILURE IN BABOON SEPTICEMIA. G. Schlag, H. Redl, A. Schießer and J. Davies*. Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria; * Roodeplaat Research Laboratories, Pretoria, South Africa.

Aim of the Study:
Monitoring of the clinical course of subchronic septicemia and evaluation of pathological findings in organ failure.
Methods:
1. Physiological organ failure baboon score (ph OFBS): Based on the physiological parameters used in the acute physiology score (APS) of the APACHE II system, we have set up a disease classification system in baboons. Similar to APS (corrected to baboon normal values) a score 0 - 4 was used for 9 parameters (e.g. temperature, arterial and pulmonary pressure, leukocytes). In addition level of consciousness and food/fluid intake was evaluated. To speed up calculation a program on a portable PC was established.
2. Morphologic organ failure baboon score (m OFBS): Post mortem findings are evaluated in lung, liver, kidney, heart, gut and adrenals. Weight parameters are evaluated only in lung and liver. Post mortem findings and weight parameters are combined with a special scoring system, which resulted in a score of 0 - 3/organ.
Conclusion:
These scoring systems allow precise follow up during chronic studies as well as a post mortem scored evaluation of organ failures.

Author-Abstract Index*

*Numbers correspond to abstract number, not page number.

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MEETINGS

National Meetings

1st June 1–3, 1978, Airlie, Virginia
William Schumer, MD, Chair
Abstracts: Circulatory Shock 5:2, 183–232, 1978
Papers: Advances in Shock Research, Vols. 1 & 2, 1979, and Metabolic and Cardiac Alterations in Shock and Trauma. Circulatory Shock, Supplement 1, 1979

2nd June 7–9, 1979, Williamsburg, Virginia
David G. Reynolds, PhD, Chair
Abstracts: Circulatory Shock 6:2, 165–198, 1979
Papers: Advances in Shock Research, Vols. 3 & 4, 1980

3rd June 11–13, 1980, Lake of the Ozarks, Missouri
Lerner B. Hinshaw, PhD, Chair
Abstracts: Circulatory Shock 7:2, 187–223, 1980
Papers: Advances in Shock Research, Vols. 5 & 6, 1981

4th June 4–6, 1981, Marco Island, Florida
Sherwood M. Reichard, PhD, Chair
Abstracts: Circulatory Shock 8:2, 1981
Papers: Advances in Shock Research, Vols. 7 & 8, 1982

5th June 9–11, 1982, Smuggler's Notch, Vermont
Robert R. Wolfe, PhD, Chair
Abstracts: Circulatory Shock 9:2, 1982
Papers: Advances in Shock Research, Vols. 9 & 10, 1983

6th June 6–8, 1983, Grand Teton National Park, Wyoming
Robert W. Phillips, PhD, Chair
Abstracts: Circulatory Shock 10:3, 1983

7th June 4–6, 1984, Toronto, Canada
Glen A. Taylor, MD, Chair
Abstracts: Circulatory Shock, 13:1, 1984

8th June 9–12, 1985, Baltimore, Maryland
Daniel L. Traber, PhD, Chair
Abstracts: Circulatory Shock, 16:1, 1985

9th June 8–11, 1986, Scottsdale, Arizona
Gerald S. Moss, MD, Chair
Abstracts: Circulatory Shock, 18:4, 1986

10th June 7–11, 1987, Montreal, Canada
Robert F. Bond, PhD, Chair
Abstracts: Circulatory Shock, 21:4, 1987

11th June 5–8, 1988, Lake Geneva, Wisconsin
John C. Passmore, PhD, Chair
Abstracts: Circulatory Shock, 24:4, 1988

12th June 9–12, 1989, Marco Island, Florida
Irshad H. Chaudry, PhD, Chair
Abstracts: Circulatory Shock, 27:4, 1989

13th June 8–11, 1990, Durango, Colorado
H. Richard Adams, DVM, PhD, Chair
Abstracts: Circulatory Shock, 31:1, 1990

INTERNATIONAL CONGRESSES

1st June 7–11, 1987, Montreal, Canada
Robert F. Bond, PhD, Chair
Abstracts: Circulatory Shock, 21:4, 1987

2nd June 2–6, 1991, Vienna, Austria
Gunther Schlag, MD, Chair
Abstracts: Circulatory Shock, 34:1, 1991

INTERNATIONAL SYMPOSIA

July 10–24, 1980, Budapest, Hungary
Cosponsors: Shock Society and International Congress of Physiology
Arisztid G.B. Kovach, John J. Spitzer, and H.B. Stoner, Chairs.
Papers: Advances in Physiological Sciences, Vol. 26, Homeostasis in Injury and Shock, Pergamon Press, 1981

September 5–8, 1984, Manchester, England. "The Scientific Basis of the Care of the Critically Ill." M.H. Irving and R.A. Little, Chairs. Partially supported by the Shock Society.

Constitution

CONSTITUTION OF THE SHOCK SOCIETY

ARTICLE I (Name)

The name of the society shall be the SHOCK SOCIETY.

ARTICLE II (Purpose)

The purpose of the Society shall be:

1. To promote original research in the fields of Shock and Trauma.

2. To provide a forum for the multidisciplinary integration of current basic and clinical knowledge and concepts in the study of shock and trauma.

3. To promote the dissemination and applications of knowledge of these fields.

4. To promote an awareness of the national and international health importance of shock and trauma.

ARTICLE III (Membership)

Membership in the Society shall be open to persons who share the stated purpose of the Society and who have educational, research, or clinical experience in the field of shock and trauma or in an allied discipline.

ARTICLE IV (Officers)

The officers of the Society shall be a President, a President-Elect, a Secretary, and a Treasurer. The President-Elect shall serve one year as such, followed by one year as President. No person shall ever be eligible for re-election to the Presidency.

The Secretary and Treasurer shall be elected to terms of two years. The Secretary will be elected on odd years and the Treasurer on even years. No person may hold the offices of Secretary and Treasurer for more than two terms.

ARTICLE V (Council)

There shall be a Council responsible for the fulfillment of the scientific and business obligations of the Society.

The current Officers, the immediate Past-President, the Editor(s) of the official Society Journal—Circulatory Shock, the Chair of the Scientific Program Committee, and six additional Councilors shall constitute this Council. Councilors shall be elected to provide representation from the various subdivisions of shock research. Councilors shall be chosen by the membership of the Society for three-year terms, two to be elected each year. No Councilors shall be eligible for re-election until one year after the expiration of a full three-year term. Upon the recommendation of the Publications Committee, the Editor(s) will be elected by the Council for a four-year term and may be immediately eligible for re-election.

ARTICLE VI (Affiliations)

The Society is empowered to affiliate with other organizations.

Proposals for affiliation may be initiated by individual Members of the Council or by a petition to the Council signed by ten Members of the Society, and to become effective must be approved by a two-thirds majority of the Council and approved by the membership.

ARTICLE VII (Bylaws)

The provisions of the Constitution of the Society shall be carried out in accordance with the current Bylaws of the Society.

ARTICLE VIII (Amendments)

Amendments may be initiated by individual Members of the Council or by a petition to the Council signed by ten Members of the Society. Amendments must be approved by a two-thirds majority of the Council, must then be discussed at a subsequent business meeting of the Society, and must finally be ratified in a mail ballot by a majority of those Members of the Society voting.

ARTICLE IX (Dissolution)

Dissolution of the Society for any cause shall be initiated by individual Members of the Council or by a petition to the Council signed by ten Members of the Society. Such motion or petition must be approved by a two-thirds majority of the Council, must then be discussed at a subsequent business meeting of the Society, and must finally be ratified in a mail ballot by two-thirds of those Members of the Society voting. Dissolution must be in accordance with the applicable regulations of the 1965

Internal Revenue Code, Section 506, or any amendments thereto.

All funds and other assets of the Society, including any rights to funds, present or future, contingent or actual, shall be irrevocably assigned and transferred to any successor society which has among its principal purposes the encouragement, development, and dissemination of knowledge in the biological or physical sciences, and has qualified as an exempt organization under Section 501 of the 1954 Internal Revenue Code. Such activities or any amendments thereto need not be the only purpose of the successor society.

The selection of the successor society must be approved by a two-thirds vote of the Council and named in the Council's minutes and its Articles of Dissolution, but need not be named in the motion of petition for dissolution.

BYLAWS*

ARTICLE I (Membership)

1. The membership of the Society shall consist of Members (including Charter Members), Student Members, Associate Members, Emeritus Members, and Sustaining Members.

2. Members. A person who shares the stated purpose of the Council and is eligible under *Article III of the* Constitution may be elected a Member. Applicants must be sponsored by two Members. Applications must be submitted to the Society office and will then be transmitted to the Membership Committee for approval.

3. Student Members. The principal requirement for Student Membership is a genuine and active interest in the aims and purposes of the Society. Applicants must be sponsored by an active member of the Society. The fee for Student Membership shall be the Society's cost of the Journal, Circulatory Shock, or 1/2 of the Society's dues without the Journal. Membership shall be renewable each year for a maximum of 5 years. Application for Full Membership in the Society is then required. Student Membership does not include voting privileges in the Society. Student Members may submit one paper at the Annual Meeting without Full member sponsorship, but may not sponsor any papers at the Annual Meeting.

4. Associate Members. The principal requirement for Associate Membership is a genuine and active interest in the aims and purposes of the Society. Applicants must be sponsored by an active member of the Society. The fee for Associate Membership shall be more than that for full members, but less than the subscription rate for nonmembers. Application for Full membership in the Society may be made whenever an appropriate degree of experience or publications has been achieved. Associate Membership does not include voting privileges in the Society. Associate Members may submit one paper at the Annual Meeting without Full member sponsorship, but may not sponsor any papers at the Annual Meeting.

5. Emeritus Members. A Member who has retired or become emeritus may apply to the Council for election to emeritus status. Emeritis Members shall pay no dues but shall have all rights and privileges of Members.

6. Sustaining Members. The Council may elect a person or corporation a Sustaining Member as a result of demonstrated and substantial acts benefitting the Society or its purposes. Only in the case of a person qualified as a Member may a Sustaining Member vote or hold office.

ARTICLE II (Meetings)

The Society is authorized to hold scientific meetings, international, national, and regional. A business meeting shall be held in connection with the annual scientific meeting of the Society. Parliamentary procedures to be followed in the business meeting shall be those specified in "Robert's Rules of Order." Five percent of the Members, or 50, whichever is smaller, shall constitute a quorum.

ARTICLE III (Dues)

All fiscal affairs of the Society shall be conducted on the basis of the calendar year.

Membership dues may be changed by the Council, subject to approval at the next annual business meeting.

Annual dues are payable on October 1st preceding the beginning of the fiscal year. Members who have not paid by December 1st will be notified and if they still have not paid by the first day of the fiscal year they will be dropped from the mailing list. Prior to the following April 1st, Members will be reinstated upon payment of dues; if in arrears on that date, they will be dropped from membership.

ARTICLE IV (Publications)

The Society is empowered to publish or to enter into agreements with others to publish such journals and other publications (abstracts, reviews, newsletters, etc.) as may be authorized by a two-thirds majority vote of the Council. Changes in the agreements which implement the publishing of a duly established journal or other organ may be authorized by a majority vote of the Council.

ARTICLE V (Duties of Officers)

It shall be the duty of the President to preside over the annual business meeting of the Society, to serve as chair of the Council, to appoint and charge, with the approval of the Council, the Chair and members of all committees of the

*Amended January, 1983; June, 1986; June, 1987; June, 1988.

Council, and to carry out other activities usually pertaining to the office.

The President-Elect shall carry out the duties of an absent or disabled President. The President-Elect will automatically succeed to the presidency when the office becomes vacant.

The Secretary shall keep accurate records, maintain an up-to-date membership list, and give notice of all meetings of members and of the Councils.

The Treasurer shall send out dues notices and collect all dues. He shall be responsible for all funds and securities of the Society, and shall make all disbursements in accordance with the budget approved by the Council. He shall submit an annual report of the financial condition of the Society and be responsible for any financial reports required by the Internal Revenue Service.

ARTICLE VI (Duties of the Council)

The duties of the Council shall be to determine the policies for the good of the Society and the science it represents in accordance with the Constitution and to implement the execution of these policies as provided in these Bylaws. It shall plan scientific meetings; it shall authorize the expenditure of Society funds, and it shall obtain an annual audit of the Society finances.

The Council shall fill a vacancy in the offices of Secretary and Treasurer until the office can be filled by a regular election of the Society; and in the event that the presidency becomes vacant when there is no President-Elect, it shall elect one of its numbers as Acting President until a regularly elected President takes office.

Interim vacancies among the Councilors may be filled by the Council until the next regular election of the Society.

Upon the recommendation of the Publications Committee, Council shall elect the Editor(s) of its official journal(s) by a two-thirds majority vote.

The Council may, if it deems necessary, appoint an Executive Secretary with appropriate compensation to assist in handling the affairs of the Society.

The Council may, at its discretion, appoint at Executive Committee from its members and may delegate to this committee such powers as it sees fit.

The Council shall meet, at the call of the President, at least once a year. At the regular meeting it shall consider changes in dues, amendments to the Constitution and Bylaws, and proposals for affiliation, and set the agenda for the business meeting. Newly elected Council members who have not yet taken office, are expected to attend this meeting, but may not vote. The Council shall have power to conduct other business by means of mail vote.

Six voting Members of the Council shall constitute a quorum.

The Council may apply for grants or secure donations for specific projects which are consistent with the purposes of the Society and they or appropriate Committees of the Council may then meet to consider their business at times other than the Annual Meeting with expenses defrayed by said grants or donations.

ARTICLE VII (Elections)

Nominations for offices to become vacant shall be made by the nominating committee. Nominations will also be received by petition. Each petition must be signed by ten Members and must contain a written statement by the nominee of willingness to serve. In order that the names of persons so nominated may appear on the ballot, petitions must be received by the Secretary before January 1st. The final list of nominees arranged as a ballot, and containing more than one name for each vacancy to be filled, shall be mailed to the Members. The candidate for each office receiving the highest number of votes will be elected.

The election of Councilors shall follow the same schedule as for the election of officers. The slate of the nominating committee shall contain at least one more name than the number of vacancies for both full and unexpired terms. Additional nominations for Councilor may be made by petition. Each petition must be signed by five Members and must contain a written statement of willingness to serve.

All officers and Councilors shall take office at the end of the annual business meeting.

ARTICLE VIII (Standing Committees)

1. Awards and Honors Committee. The Awards and Honors Committee shall normally be composed of three members, two of whom are Past-Presidents of the Society. Each President appoints one member to a three-year term and designates the Chair of the Committee. The Committee is charged with the responsibility for selecting finalists from the abstracts entered by students in training (Predoctoral or Postdoctoral). Finalists will present their work at the Annual Meeting. The Committee may also be charged with selecting a member of the Society who has shown consistent excellence in research. The award will be a named award. Any recommendation for new awards and honors made by the Council or membership will be referred to this Committee for discussion and recommendation. This Committee can also initiate recommendations and other ideas for Awards and Honors appropriate to the goals and objectives of the Society.

2. Development Committee. The Chair of the Development Committee shall be appointed for a three-year term and shall be a member of the Finance Committee. The Chair, with the consent of the President, may appoint additional members to the Committee as needed. The Development Committee is responsible for (1) developing plans for the Society over the next few years, (2)

coordinating Society activities affecting corporations, (3) soliciting sustaining members, (4) recommending benefits for sustaining members, (5) coordinating the solicitation of sponsors of workshops and symposia at the Annual Meeting, (6) soliciting exhibits for the Annual Meeting, and (7) improving communication between the private sector and the Society.

3. The Finance Committee. The Finance Committee shall be composed of the Treasurer (as Chair), the Chair of the Development Committee, and the President-Elect. The administrative officer of the Society may serve as an ex-officio member of this Committee. The Committee shall prepare an annual Society budget and submit it for Council approval at the time of the Annual Meeting and prior to the start of the fiscal year. This budget shall include estimates of all income sources, and appropriate estimates of expenditures for Committees, Officers, Meetings, and a Publications Operating Fund may be established upon Council approval. The Finance Committee shall consider and attempt to devise ways to increase the Society's income.

4. International Relations Committee. The International Relations Committee shall be composed of three members elected by Council from among four nominees submitted by the President. Their terms of office shall be for three years, one being elected each year. The President shall designate one member of the Committee to serve as Chair. Members of this Committee shall be the official delegates to any International Meeting and be responsible for the foreign activities of the Society.

5. Membership Committee. The Membership Committee shall be composed of three members, each serving a term of three years. The primary purposes of the Committee are to increase individual memberships in the Society and to review applications for membership. Applicants may be granted membership by the Committee. Applications must attain an aproval vote of at least two-thirds of the Committee.

6. Nominating Committee. The Nominating Committee shall be composed of the immediate Past-President who will be Chair of the Committee and at least three other members of Council appointed by the President, each serving three years. Committee members may not currently be from the same institution. The Nominating Committee shall submit nominations of the offices of President-Elect, Secretary, and Treasurer. They shall also submit the names of at least two members of the Society as candidates for each position of Councilor and two members for each position on the Scientific Program Committee. It will be the responsibility of the Nominating Committee to prepare lists of nominees from the members as described in Article VII of the Bylaws and to ascertain the willingness of each nominee, if elected, to serve. The Committee transmits nominations to the Secretary for publication at least six months prior to the Annual Meeting. Other names may be added to the Ballot upon petition in accordance with the procedures published in Article VII of the Bylaws. At least 3 months before the Annual Meeting, a Ballot containing the list of all nominees will be sent to the membership. For a member to be eligible for nomination for elective office, he/she must be an active member in good standing for a minimum of two years.

7. Publications Committee. The Publications Committee shall be composed of four members appointed by the President, each serving four years, one being appointed each year. The senior member will be the Chair. The Society Editor serves in a non-voting capacity. The Committee formulates general policy concerning all publications and makes decisions concerning publications arising out of Annual and International Meetings, subject to review and approval by the Council. The Committee is responsible for nominating an Editor(s) for Council approval. The Committee serves as a liaison between the membership and the Journal, offering advice and comment on general publication policy.

8. Rules Committee. The Rules Committee shall be composed of one member appointed each year by the President who will serve as Chair. The Chair may appoint additional members as needed. The Chair of this Committee shall serve for a term of one year and may be reappointed. The Chair of the Committee becomes the Parliamentarian of the Society with such duties as may be set forth in the Bylaws or Rules of the Society. Questions relative to the interpretation of the Constitution shall be presented to the Rules Committee. The duties of this Committee shall be to provide information for the Council on matters relating to the Constitution of the Society, its Bylaws, and acts of the Annual Meeting; to interpret for the Council the Constitution, Bylaws, and acts of the Annual Meeting; to recommend to the Council the requirements for, and privileges and obligations of, the several classes of membership; and to consider from time to time, either on its own initiative or by reference from the Council or the Membership, proposed revisions of the Constitutions and Bylaws.

9. Scientific Program Committee. The Scientific Program Committee shall be composed of six members, representing the present and next two Annual Meetings, three elected members and three members appointed by the elected members. Elected members shall each serve three years, one being elected each year. Elected members shall be nominated by the Nominating Committee and these nominees should represent the scientific interests of the Society.

The Scientific Program Committee is responsible for the scientific affairs of the Society. The Committee develops the program for the Annual Meeting, including topics and contributors for major sessions and selection of proffered papers. It arranges for the program publication and receives proposals and makes recommendations to

Council concerning selection and scheduling of sites for Annual Meetings. Further, the Committee is responsible for scientific programs held in cooperation with other American organizations. The Committee is required to file a formal written summary annually with the Council.

10. Laboratory Animal Issues Committee. The Laboratory Animal Issues Committee shall be composed of four appointed members, three of whom shall serve for a term of three years, one being appointed each year by the President. The Chair of this Committee shall serve for a term of one year, and may be reappointed. The purpose of this Committee is to: 1) promote the ethical and humane use of laboratory animals as required for legitimate scientific research, 2) gather and provide the membership with current information concerning matters that could affect the Society's purpose of promoting research in shock and trauma. Such matters might include the status of pending legislation dealing with animal care or use, the activities of animal activist groups, and national efforts to foster biomedical research. 3) In collaboration with the Program Committee, sponsor appropriate programs at the annual meetings.

ARTICLE IX (Amendments)

Amendments to the Bylaws shall be initiated according to the same procedure as amendments to the Constitution, except that a majority vote at the annual business meeting shall suffice for ratification.

Membership Directory

Abel, Francis L.
Dept. of Physiology
Univ. South Carolina Sch. Med.
Columbia, SC 29208

Abraham, Edward
Dept. of Med.
Div. Pulmonary and CCM
UCLA Medical Ctr.
Los Angeles, CA 90024

Abumrad, Naji N.
Dept. of Surgery
Vanderbilt Univ.
A2219 MCN Vanderbilt Med. Ctr.
Nashville, TN 37232

Adams, H. Richard
Dept. of Biomedical Sciences
Univ. Missouri-Columbia
Col. of Vet. Med.
Columbia, MO 65211

Al Tuwaijri, Ali S.
Dept. of Physiology
King Saud Med. Sch.
PO Box 2925
Riyadh, Saudi Arabia 11461

Albina, Jorge E.
Dept. of Surgery
Rhode Island Hosp.
593 Eddy St.
Providence, RI 02903

Alexander, J. Wesley
Dept. of Surgery
Univ. Cincinnati Col. Med.
231 Bethesda
Cincinnati, OH 45267

Allen, Elizabeth J.**
1305 Westgate Terrace
Chicago, IL 60607

Allo, Maria D.
Dept. of Surgery-Osler 624
Johns Hopkins Hosp.
600 N. Wolfe St.
Baltimore, MD 21205

Alteveer, Robert J.
Physiology & Biophysics
Hahnemann Univ. Sch. Med.
Philadelphia, PA 19102

Altura, Burton M.
Dept. of Physiology/Box 31
SUNY Downstate Med. Ctr.
450 Clarkson Ave.
Brooklyn, NY 11203

Alverdy, John C.
Dept. of Surgery
Michael Reese Hosp.
Lake Shore Drive at 31st St.
Chicago, IL 60616

Amaral, Joseph F.
Dept. of Surgery
Rhode Island Hosp.
593 Eddy St.
Providence, RI 02902

Antonenko, David R.
501 Columbia Rd.
Grand Forks, ND 58203

Applefeld, Jack J.**
Critical Care
Good Samaritan Med. Ctr.
1111 E. McDowell Rd.
Phoenix, AZ 85006

*Emeritus Members
**Associate Members
***Student Members

Archer, Linda T.
VA Med. Ctr.
Lab Service (113)
921 N.E. 13th St.
Oklahoma City, OK 73104

Arfors, Karl E.
Pharmacia Experimental Med.
11099 N. Torrey Pines Rd.
La Jolla, CA 92037

Asher, Eleanor F.
953 Cherokee Rd.
Louisville, KY 40204

Ayala, Alfred
Dept. of Surgery
Michigan State Univ.
B409 Clinical Ctr.
East Lansing, MI 48824

Babbs, Charles F.
Biomed. Eng. Ctr.
Purdue Univ.
Potter Bldg.
W. Lafayette, IN 47907

Bagby, Gregory J.
Dept. of Physiology
Louisiana State Med. Ctr.
1901 Perdido
New Orleans, LA 70112

Bajo, Thomas**
Critical Care
Good Samaritan Med. Ctr.
1111 E. McDowell
Phoenix, AZ 85006

Baker, Carleton H.
Dept. of Physiology
Univ. South Florida
Col. Med./Box 8
Tampa, FL 33612

Baker, Christopher
Dept. of Surgery
Univ. North Carolina
215 Burnett Womack Bldg.
Chapel Hill, NC 27599

Baker, Robert J.
Dept. of Surgery
Med. Ctr. of Delaware
501 W. 14th St.
Wilmington, DE 19899

Balis, John U.
Dept. of Pathology/Box 11
Univ. South Florida Col. Med.
12901 N. 30th St.
Tampa, FL 33612

Ball, Howard A.
Ciba Geigy
K.125.9.12
Basel, Switzerland CH-4002

Barie, Philip S.
Dept. of Surgery
Cornell Univ.
525 E. 68th St., F-1926
New York, NY 10021

Barillo, David J.
Dept. of Surg./Burn Unit
Lehigh Valley Hosp.
1230 S. Cedar Crest #303
Allentown, PA 10103

Barker, Geoffrey**
Dept. of Critical Care
Hospital for Sick Children
555 University Ave.
Toronto, Ont., Canada M5G 1X8

Barker, Louis A.
Dept. of Pharmacology
LSU Med. Ctr.
1901 Perdido St.
New Orleans, LA 70112

Barrett, John A.
Trauma Office, M-3241
County Hosp.
1835 W. Harrison
Chicago, IL 60612

Baue, Arthur E.
Vice President Med. Ctr.
St. Louis Univ. Med. Ctr.
3556 Caroline St.
St. Louis, MO 63104

Beamer, Kathryn C.
Dept. of Surgery
West Virginia Univ.
Sch. Med.
Morgantown, WV 26505

Benjamin, Ernest
Falk ICU
Mount Sinai Hosp.
1 Gustave Levy Place
New York, NY 10029

Bessey, Palmer Q.
Dept. of Surgery
Washington Univ. Sch. Med.
Box 8109
St. Louis, MO 63110

Biber, Bjorn
Dept. of Anesthesiology
Ostra Hosp.
Gothenburg, Sweden S 41685

Bitterman, Haim
Dept. of Med. A
Lady Davis Carmel Hosp.
7 Michal St.
Haifa, Israel 34362

Blackwood, James M.
Dept. of Surgery/G532
New Jersey Med. Sch.
100 Bergen St.
Newark, NJ 07103

Blaisdell, F. William
Dept. of Surgery
Univ. California Davis
4301 X St.
Sacramento, CA 95817

Blumenstock, Frank A.
Dept. of Physiology
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Bond, Robert F.
Dept. of Physiology
Univ. South Carolina
Sch. Med.
Columbia, SC 29208

Borlase, Bradley C.**
Dept. of Surgery
New England Deaconess
110 Francis St.
Boston, MA 02215

Botan, Edward A.
A.P.P. Research Cardiosurgery
C.R. Bard Inc.
129 Concord Rd./P.O. Box M
Billerica, MA 01821

Bottoms, Gerald D.
Dept. of Veterinary
Physiol. & Pharmacol.
Purdue Univ.
W. Lafayette, IN 47907

Bowen, John C.
Surgical Education
Ochsner Med. Inst.
1514 Jefferson Highway
New Orleans, LA 70121

Boyd, John L., III***
Dept. of Pediatrics
Duke Univ. Med. Ctr.
P.O. Box 3046
Durham, NC 27705

Brackett, Daniel J.
Research Service (151)
V.A. Med. Ctr.
921 N.E. 13th St.
Oklahoma City, OK 73104

Breslow, Michael J.***
7 Broadridge Lane
Lutherville, MD 21093

Brinson, Robert R.
Internal Med.
Baptist Med. Ctr.
2055 E. South Blvd./Suite 706
Montgomery, AL 36116

Britt, L. Delano**
Dept. of Surgery
Eastern Virginia Med. Sch.
825 Fairfax Ave.
Norfolk, VA 23507

Brotman, Sheldon
Dept. of General Surgery
Geisinger Med. Ctr.
Danville, PA 17821

Buchman, Timothy G.**
Dept. of Surgery
Johns Hopkins
600 N. Wolfe St.
Baltimore, MD 21205

Buehren, Volker**
Dept. of Trauma-Surg.
Univ. Saarland
Homburg/Saar, Germany 6650

Bulkley, Gregory B.
Dept. of Surgery
Johns Hopkins Univ.
600 N. Wolfe St.
Baltimore, MD 21205

Burchard, Kenneth W.
General Surgery
Hitchcock Clinic
2 Maynard St.
Hanover, NH 03756

Burhop, Kenneth E.
Dept. of Physiology
Baxter Healthcare
Baxter Technology Park, WG2-1S
Round Lake, IL 60073

Burke, John F.
Dept. of Surgery
Massachusetts Gen. Hosp.
Fruit St.
Boston, MA 02114

Burns, J. Robert
Critical Care Med.
Geisinger Med. Ctr.
Danville, PA 17821

Caffrey, James L.
Dept. of Physiology
Texas Col. Osteopathic Med.
3500 Camp Bowie Blvd.
Ft. Worth, TX 76107

Cain, Stephen M.
Prof. Physiol. & Biophys.
Univ. Alabama, Birmingham
UAB Station, 401 VH
Birmingham, AL 35294

Caldwell, Michael D.
Dept. of Surgery
Rhode Island Hosp.
Providence, RI 02902

Canada, Andrew T.**
Dept. of Anesthesiology
Duke Univ. Med. Ctr.
Box 3094
Durham, NC 22710

Cane, Roy D.
Dept. of Anesthesiology
Univ. South Florida
Box 59, 12901 B Downs Blvd.
Tampa, FL 33612

Canizaro, Hana P.
Dept. of Surgery
Texas Tech. Univ.
Hlth. Sci. Ctr.
Lubbock, TX 79430

Carli, Alain
U Cochin Port Royal
Serv. Reanim. Polyvalente
27 Rue FBG St. Jacques
Paris, France 75674

Carmona, Richard
Surgery Trauma Services
Tucson Med. Ctr./P.O. Box 42195
Tucson, AZ 85733

Carrico, C. James
Dept. of Surgery
RF-25
Univ. Washington
Seattle, WA 98195

Carroll, Gilbert C.
44946 Cougar Circle
Fremont, CA 94539

Carroll, Robert G.
Dept. of Physiology
East Carolina Univ. Sch. Med.
Greenville, NC 27858

Carvajal, Hugo F.
Dept. of Pediatrics
Univ. of Texas Med. Sch.
6431 Fannin
Houston, TX 77030

Casey, Kenneth F.
Room L 1092
263 Farmington Ave.
Farmington, CT 06032

Castillo, Leticia**
Pediatric Intensive Care
Mass. General Hosp.
Ellison 3 MGH/32 Fruit St.
Boston, MA 02114

Cavanagh, Denis
Dept. of Ob/Gyn.
U. So. Fla. at Moffitt Cancer Ctr.
12902 Magnolia Dr.
Tampa, FL 33612

Cerra, Frank B.
Box 42, Mayo Bldg.
Univ. Minnesota
420 Delaware St., S.E.
Minneapolis, MN 55455

Chaudry, Irshad H.
Dept. of Surgery
B424 Clinical Ctr.
Michigan State Univ.
East Lansing, MI 48824

Chen, Hua-Cui
Dept. of Pathophysiology
Hsaing-Ya Med. Col.
5 Dong Dan San Tiao
Beijing, China

Chernow, Bart
Sinai Hosp. of Baltimore
Belvedere at Greenspring
Baltimore, MD 21215

Chiu, Ray Chu-Jeng
Dept. of Surgery
Montreal General Hosp.
1650 Cedar Ave.
Montreal, Quebec, Canada H3G1A4

Cho, Eshin
Dept. of Physiology, A134
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Cilley, Robert E.
Pediatric Surgery
Univ. Michigan
F7516 Mott Children's Hospital
Ann Arbor, MI 48109

Clemens, Mark G.
Dept. of Surgery (Pediatric)
Johns Hopkins Univ. Sch. Med.
600 N. Wolfe St.
CMSC 7-121
Baltimore, MD 21205

Cocanour, Christine S.**
Dept. of Surgery
U.T.H.S.C. Houston
6431 Fannin MSB 4276
Houston, TX 77030

Cohn, Stephen M.
Dept. of Surgery
Yale Sch. Med.
333 Cedar St.
New Haven, CT 06510

Colucci, Robert D.
30 Country Club Blvd.
Scotch Plains, NJ 07076

Cone, John B.
Dept. of Surgery
Univ. Arkansas
4301 W. Markham
Little Rock, AR 72205

Connell, Reid S.
Dept. of Anatomy
OHSU
3181 S.W. Sam Jackson Park Rd.
Portland, OR 97201

Conrad, Steven A.**
Dept. of Medicine
LSU Med. Ctr.
1501 Kings Highway
Shreveport, LA 71130

Cook, James A.
Dept. of Physiology
Med. Univ. South Carolina
171 Ashley Ave.
Charleston, SC 29425

Coran, Arnold G.
Pediatric Surgery
Univ. Michigan
Mott Children's Hosp.
Ann Arbor, MI 48109

Cornell, Robert P.
Div. of Science
Northeast Missouri State Univ.
Kirksville, MO 63501

Cowley, R. Adams
Maryland Inst. for EMS
Univ. Maryland
22 S. Greene St.
Baltimore, MD 21201

Croce, Martin A.
Surgery
Univ. Tennessee
956 Court Ave., Suite E-228
Memphis, TN 38163

Cronen, Paul
703-A Green Rd.
Madison, IN 47250

Cryer, H. Gill
UCLA Div. of Gen. Surg.
10833 Le Conte Ave.
77-130-CHS
Los Angeles, CA 90024

Cunningham, Paul
Dept. of Surgery
East Carolina Univ.
PCM H, Room 204
Greenville, NC 27858

Dahn, Michael S.
Univ. Health Ctr./6C
4201 St. Antoine
Detroit, MI 48201

Dawidson, Ingemar J.A.
Dept. of Surgery
Southwestern Med. Sch.
5323 Harry Hines Blvd.
Dallas, TX 75235

Dehring, Deborah J.
Dept. of Anesthesiology
Univ. Texas Med. Branch
E91
Galveston, TX 77550

Deitch, Edwin
Dept. of Surgery
LSU Med. Ctr.
P.O. Box 33932
Shreveport, PA 71130

Demetriou, Achilles
Dept. of Surgery
B-306 VAMC
1310 24th Ave. S.
Nashville, TN 37212

Demling, Robert H.
Longwood Area
Trauma Ctr.
75 Francis St.
Boston, MA 02115

Didlake, Ralph
Dept. of Surgery
Univ. Miss. Med. Ctr.
2500 N. State St.
Jackson, MS 39216

Doran, Jan Eva
Dept. of Experimental Med.
Swiss Red Cross, Central Lab.
Wankdorfstrasse 10
3000 Bern 22, Switzerland

Dries, David J.
Dept. of Surgery
Loyola Med. Ctr.
2160 S. First Ave.
Maywood, IL 60153

Dulchavsky, Scott A.
Dept. of Surgery
SUNY at Stony Brook
Health Sci. Ctr. T19060
Stony Brook, NY 11794

Dunham, C. Michael
Dept. of Traumatology
MIEMSS—Univ. Maryland
22 S. Greene St.
Baltimore, MD 21201

Durkot, Michael John
Dept. of the Army
USATIEM
Comparative Physiology Div.
Natick, MA 01760

Dyess, Donna Lynn**
Dept. of Surgery
Univ. Alabama
222 CSAB
Mobile, AL 36688

Ebata, Toshiaki
Dept. of Surgery
Sapporo Med. Col.
S-1, W-16, Chuo-Ku
Sapporo, Japan 060

Emerson, Thomas E.
Dept. of Physiology
Cutter Group of Miles Labs. Inc.
Fourth & Parker Sts./Bld28A
Berkeley, CA 94710

Enderson, Blaine L.
Dept. of Surgery
Univ. Tennessee Med. Ctr.
1924 Alcoa Highway
Knoxville, TN 37920

Engquist, Allan
Dept. of Int. Care Therapy
Bispebjerg Hosp.
Copenhagen, Denmark 2400

Enzan, Keiji**
Dept. of Anesthesiology
Akita Univ. Sch. Med.
1-1-1 Hondo
Akita 010, Japan

Ephgrave, Kimberly S.
Dept. of Surgery
Univ. Iowa Col. Med.
Iowa City, IA 52240

Ertel, Wolfgang
Chirurgische Klinik & Poiklinik
Der Ludwig Maximilians Univ.
Marchioninistrasse 15
Munich 70, Germany D8000

Fabian, Miklos
Univ. Maryland
RD #3/Box 308
Delta, PA 17314

Fabian, Timothy C.
Dept. of Surgery
Univ. Tennessee
956 Court Ave.
Memphis, TN 38163

Fagraeus, Lennart
Dept. of Anesthesiology
Christiana Hosp.
P.O. Box 6001
4755 Ogletown-Stanton Rd.
Newark, DE 19718

Fantini, Gary A.
Dept. of Surgery
N.Y. Hosp./Cornell Med. Ctr.
525 E. 68th St./Suite 1920
New York, NY 10021

Feola, Mario
Dept. of Surgery
Texas Tech. Univ.
Health Sciences Ctr.
Lubbock, TX 79430

Ferguson, James L.
Physiol. & Biophys.
Univ. Illinois at Chicago
835 S. Wolcott, CMHSA M/C 901
Chicago, IL 60612

Fessler, John F.
Dept. of Large Animal Clinics
Sch. Veterinary Med.
Purdue Univ.
W. Lafayette, IN 47907

Fettman, Martin J.
Dept. of Pathology
Colorado State Univ.
Col. Veterinary Med.
Ft. Collins, CO 80523

Feuerstein, Giora Z.
Director, Cardiovascular Pharm.
Smith Kline Beecham
P.O. Box 1539, L-524
King of Prussia, PA 19406

Fiddian-Green, Richard
Gen. Surgery
Univ. Mass. Med. Ctr.
55 Lake Ave.
Worcester, MA 01655

Fildes, John**
Trauma Unit
Cook County Hosp.
1835 W. Harrison St.
Chicago, IL 60612

Filkins, James P.
Dept. of Physiology
Loyola Univ. Med. Ctr.
2160 S. First Ave.
Maywood, IL 60153

Fink, Mitchell P.
Dept. of Surgery
Univ. Mass. Med. Ctr.
55 Lake Ave. North
Worcester, MA 01655

Fischer, Ronald P.
Dept. of Surgery
Univ. Texas Med. Sch.
6431 Fannin
Houston, TX 77030

Fish, Richard E.
Dept. of Lab. Animal Med.
Univ. Missouri
M144 Med. Sci. Bldg.
Columbia, MO 65212

Flancbaum, Louis J.
Dept. of Surgery, N737 Doan Hall
Ohio State Univ.
410 W. 10th Ave.
Columbus, OH 43210

Fletcher, John Raymond
Dept. of Surgery
Univ. South Alabama Med. Ctr.
2451 Fillingim St.
Mobile, AL 36617

Flint, Lewis M.
Dept. of Surgery
Tulane Univ. Sch. Med.
1430 Tulane Ave.
New Orleans, LA 70112

Flye, M. Wayne
Dept. of Surgery
Washington Univ.
One Barnes Hosp. Pl. #5108
St. Louis, MO 63110

Flynn, John T.
Dept. of Physiology
Jefferson Med. Col.
1020 Locust St.
Philadelphia, PA 19107

Flynn, Timothy C.
Dept. of Surgery (112)
V.A. Med. Ctr.
Gainesville, FL 32602

Foca, Alfredo
Via Reggio Campi, 45 (1 TR)
Reggio
Calabria, Italy 89100

Fortune, John B.
Dept. of Surgery, A-61
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Franceschi, Dido**
Dept. of Surgery
Case Western Reserve
2743 Devon Hill Rd.
Rocky River, OH 44116

Frei, Lonnie**
Dept. of Surgery
SUNY at Stony Brook
HSC T-027
Stony Brook, NY 11794

Fry, Donald E.
Dept. of Surgery
Univ. New Mexico Sch. Med.
2211 Lomas, N.E.
Albuquerque, NM 87131

Furste, Wesley L.
Dept. of Surgery
Ohio State Univ.
3545 Olentangy River Rd. #126
Columbus, OH 43214

Gaffin, Stephen L.
Dept. of Physiology
Univ. Natal Med. Sch.
P.O. Box 17 039
4013 Durban, South Africa

Gamelli, Richard L.
Dept. of Surgery
Loyola Univ. Med. Ctr.
2160 S. 1st Ave./Bldg. 54, Rm. 260
Maywood, IL 60153

Gann, Donald S.
Prof. of Surgery
Univ. Maryland Med. Sch.
22 S. Greene St.
Baltimore, MD 21201

Gao, Guangcheng***
Depts. of Toxicology & Pharmacol.
Univ. Calif., Berkeley
211 Warren Hall, Sch. Public Hlth.
Berkeley, CA 94720

Garcia-Barrenc, Pedro
Service Exper. Med. and Surg.
Hosp. Provincal de Madrid
c/o Dr. Esquerdo
46 Madrid–30, Spain

Garrison, Richard N.
Dept. of Surgery
Univ. Louisville Sch. Med.
550 S. Jackson St.
Louisville, KY 40292

Geelhoed, Glenn W.
Dept. of Surgery
George Washington Univ.
2150 Pennsylvania Ave.
Washington, DC 20037

Geer, Ralph T.
Dept. of Anesthesia
Hosp. Univ. Pennsylvania
3400 Spruce St.
Philadelphia, PA 19104

Geiser, Ronald W.
Upjohn International, Inc.
Kalamazoo, MI 49001

Geller, Evan R.
Dept. of Surgery
SUNY at Stony Brook
Sch. Med., HSC19–060
Stony Brook, NY 11794

Gentili, David R.
Apartado 66145
Caracas 1061A Venezuela

Gervin, Alfred S.
Dept. of Surgery
Med. Col. Virginia
MCV Box 478-MCV Station
Richmond, VA 23298

Giovannini, Ivo
Via Alessandro VII, No 45
Rome, Italy I-00167

Glenn, Thomas M.
Biocryst, Inc.
1075 13th St. South
Birmingham, AL 35205

Goldfarb, I. William
4815 Liberty Ave.
Suite 340
Pittsburgh, PA 15224

Goldfarb, Roy D.
Dept. of Physiology
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Goodwin, Cleon W.
Burn Ctr.–Rm. F2314
NY Hosp.–Cornell Med. Ctr.
525 E. 68th St.
New York, NY 10021

Goris, Jan R.
Dept. of Gen. Surg.
St. Radboud Univ. Hosp.
P.O. Box 9101
Nijmegen, Netherlands

Goto, Masakatsu
Dept. of Pediatrics
Loyola Univ. Stritch Sch. Med.
2160 S. First Ave.
Maywood, IL 60153

Gould, Steven A.
Dept. of Surgery
Michael Reese Hosp.
Lake Shore Dr. at 31st St.
Chicago, IL 60616

Green, Douglas R.
Cellular Immunology
La Jolla Inst. Allergy & Immuno.
11149 N. Torrey Pines Rd.
La Jolla, CA 92037

Greenburg, A. Gerson
Surgery
The Miriam Hosp.
164 Summit Ave.
Providence, RI 02906

Greenfield, Lazar J.
Univ. Michigan
2101 Taubman Health Care Ctr.
Ann Arbor, MI 48109

Griffin, Andrew J.
Dept. of Pediatrics
Illinois Masonic Med. Ctr.
836 W. Wellington Ave.
Chicago, IL 60657

Griffin, David W.***
347 Overlook Dr.
W. Lafayette, IN 47906

Grindlinger, Gene A.
Critical Care Admin.
The University Hosp.
88 E. Newton St.
Boston, MA 02118

Groff, Diller B.
Dept. of Surgery
Univ. Louisville
Children's Hosp.
Louisville, KY 40232

Guice, Karen S.
Dept. of Surgery
Univ. Michigan
Box 0331, Taubman Hlth. Care Ctr.
Ann Arbor, MI 48109

Gumbs, Milton A.
The Bronx Lebanon Hosp. Ctr.
1650 Grand Concourse
Bronx, NY 10457

Gunther, Robert A.
Dept. of Surgery
Sch. Med.
4301 X St.
Sacramento, CA 95817

Gurll, Nelson J.
Dept. of Surgery
Univ. Iowa Col. Med.
Iowa City, IA 52242

Haglind, Eva
Dept. of Surgery I
Univ. Goteborg
Sahlgren's Hosp.
Goteborg, Sweden S-41345

Haglund, Ulf
Dept. of Surgery
Uppsala Univ. Hosp.
Uppsala, Sweden S-18 66

Halevy, Simon
Anesthesiology
Nassau County Med. Ctr.
East Meadow, NY 11554

Haljamae, Hengo
Dept. of Anesthesiology
Sahlgren's Hosp.
Univ. Goteborg
Goteborg, Sweden S-41345

Hall, Edward L.***
900 Gordon Ave.
Thomasville, GA 31792

Hall, John R.
Dept. of Pediatric Surgery
Cook County Children's Hosp.
Room B-40, 700 South Wood St.
Chicago, IL 60612

Halpern, Neil A.***
Dept. of Surgery Anesthesia
Bronx V.A. Med. Ctr.
130 W. Kingsbridge Rd.
Bronx, NY 10468

Halushka, Perry V.
Dept. of Pharmacology
Med. Univ. South Carolina
171 Ashley Ave.
Charleston, SC 29425

Hamar, Janos
Exp. Research Lab.
Nat. Inst. Traumatology
Mezo Imre UT 17
1081 Budapest, Hungary

Hamburger, Steven A.
Dept. of Pharmacology L510
Smith-Kline Beecham
P.O. Box 1539
King of Prussia, PA 19406

Hansbrough, John F.
Dept. of Surgery
Univ. California, San Diego
225 Dickinson St., H64OB
San Diego, CA 92101

Harbour, Deborah V.**
Dept. of Psychiatry
Univ. Texas Med. Branch
D29
Galveston, TX 77550

Harkema, James M.
Dept. of Surgery
Michigan State Univ.
B4112 Clinical Ctr.
East Lansing, MI 48824

Harlan, John M.
Dept. of Medicine/Hematology
Univ. Washington
325 Ninth Ave. ZA-34
Seattle, WA 98104

Harmon, John W.
Chief Surgical Service
Washington VA Med. Ctr.
Washington, D.C. 20422

Harris, Patrick D.
Dept. of Physiology and Biophysics
Univ. Louisville
Health Sciences Ctr. A-1115
Louisville, KY 40292

Harrison, Marvin W.
Div. of Pediatric Surgery
Oregon Health Sciences Univ.
3181 S.W. Sam Jackson Park Rd.
Portland, OR 97201

Hasselgren, Per-Olof J.
Dept. of Surgery
Univ. Cincinnati
231 Bethesda Ave.
Cincinnati, OH 45267

Haupt, Marilyn T.
Med.–Detroit Rec. Hosp.
Wayne State Univ.
4201 St. Antoine
Detroit, MI 48201

Hauptman, Joe
Small Animal Clinical Sciences
Michigan State Univ.
Veterinary Clinical Ctr.
East Lansing, MI 48824

Hay, John B.
Dept. of Immunology
Univ. Toronto
Med. Sciences Bldg.
Toronto, Ontario, Canada M5S 1A8

Hayasaka, Hiroshi
Sapporo Med. Col.
S-1, W-16
Sapporo
Hokkaido, Japan

Hechtman, Herbert B.
Dept. of Surgery
Brigham and Women's Hosp.
75 Francis St.
Boston, MA 02115

Hedlund, Bo E.
Biomed. Frontiers, Inc.
1095 10th Ave. S.E.
Minneapolis, MN 55414

Herman, Clifford M.
Dept. Surgery ZA-16
Harborview Med. Ctr.
325 9th Ave.
Seattle, WA 98104

Herndon, David N.
Shriners Burns Inst.
610 Texas Ave.
Galveston, TX 77550

Herron, David K.
Dept. of Medicinal Chem.
Lilly Research Labs
Lilly Corp. Ctr.
Indianapolis, IN 46285

Hess, Michael L.
Dept. of Physiology & Med.
Med. Col. Virginia
Box 281, MCV Station
Richmond, VA 23298

Hinshaw, Daniel B.
Dept. of Surgery (112)
V.A. Hosp.
2215 Fuller Rd.
Ann Arbor, MI 48105

Hinshaw, Lerner B.
Oklahoma Med. Research Fndn.
825 N.E. 13th St.
Oklahoma City, OK 73104

Hinson, Douglas
10335 Champions Way
Laurel, MD 20723

Hirasawa, Hiroyuki
Dept. of Emerg. & Crit. Care Med.
Chiba Univ. Sch. Med.
1-8-1 Inohana
Chiba, Japan

Hirsch, Leroy J.
Dept. of Anesthesiology
Loyola Univ. Med. Ctr.
2160 First Ave.
Maywood, IL 60153

Hirvela, Elsa R.**
Dept. of Surgery
St. Mary Hospital
56 Franklin St.
Waterbury, CT 06706

Hock, Carl E.
UMDNJ-SOM
Dept. of Med., Research Div.
401 Haddon Ave.
Camden, NJ 08103

Hoffman, James P.
Prof. Communications
Merck Sharp & Dohme
West Point, PA 19486

Holaday, John W.
Medicis Pharm. Corp.
Suite 1500
100 E. 42nd St.
New York, NY 10017

Holcroft, James W.
Dept. of Surgery
Univ. California, Davis
4301 X St./R 257
Sacramento, CA 95817

Horowitz, Bernard
New York Blood Ctr.
310 E. 67th St.
New York, NY 10021

Horpacsy, Geza
Inst. Experimental Med.
Univ. Cologne
Robert-Koch St. 10
Koln 41, Germany D-5000

Horton, Jureta
Dept. of Surgery (9031)
Univ. Texas Health Sci. Ctr.
5323 Harry Hines
Dallas, TX 75235

Hotchkiss, Richard
Dept. of Anesthesiology
Washington Univ.
14598 Ansonborugh Ct.
Chesterfield, MO 63014

Houtchens, Bruce A.
Dept. of Surgery
UTHSCH/Suite 4274
6431 Fannin
Houston, TX 77030

Hoyt, David B.
Dept. of Surgery
UCSD Med. Ctr.
225 Dickinson St., H-640B
San Diego, CA 92103

Hubbard, Joel D.
Dept. of Clinical Lab. Sci.
School of Allied Health
Texas Tech., Univ. Hlth Sciences
Lubbock, TX 79430

Hurley, R. Morrison
Dept. of Pediatrics
Loyola Univ. Med. Ctr.
2160 S. 1st Ave.
Maywood, IL 60153

Iba, Toshiaki**
Dept. of Surgery
Yale Univ.
333 Cedar St., FMB 137
New Haven, CT 06510

Iberti, Thomas J.
Surgical Intensive Care Unit
Box 1062, Mount Sinai Hosp.
1 East 100 St.
New York, NY 10029

Imai, Takasuke
Intensive Care Unit
Gunma Univ. Sch. of Med.
3-39 Showa-Machi
Maebashi 371, Japan

Ishida, Kimiko
Dept. of Gynecology & Obstetrics
Hokkaido Univ. Sch. Med.
North 14, West 5, Sapporo
Hokkaido 060, Japan

Jabs, Clarence M.
Dept. of Surgery
Kuwait Univ., Fac. Med.
PO Box 24923
Safat, Kuwait 13110

Jacobs, Donald M.**
Dept. of Surgery
Hennepin County Med. Ctr.
701 Park Ave.
Minneapolis, MN 55415

Jain, Krishna M.
Advanced Vascular Surgery
Suite 110
1535 Gull Rd.
Kalamazoo, MI 49001

James, Paul M., Jr.
415 Vernon Rd.
Jenkintown, PA 19046

Janssen, Herbert F.
Dept. of Orthopaedic Surgery
Texas Tech. Univ.
Health Sci. Ctr.
Lubbock, TX 79430

Jin, Huiming
Dept. of Pathophysiology
Shanghai Med. Univ.
138 Yi Xue Yuan Rd.
Shanghai, China 200032

Johnson, Martha A.**
Vet. Physiology & Pharm.
Purdue Univ.
Lynn Hall, Sch. of Vet. Med.
W. Lafayette, IN 47907

Johnson, Gerald, III
Dept. of Physiology
Jefferson Med. Col.
1020 Locust St.
Philadelphia, PA 19107

Jones, Stephen
Dept. of Physiology
Loyola Univ. of Chicago
Sch. Med.
2160 S. First Ave.
Maywood, IL 60153

Jones, William G.
Dept. of Surgery
Cornell Univ. Med. Ctr.
525 E. 68th, Room F–739
New York, NY 10021

Joyce, Harry H.
2819 Coachlite
Portage, MI 49081

Jurkovich, Gregory J.
Dept. of Surgery
Harborview Med. Ctr.
325 9th Ave., ZA-16
Seattle, WA 98104

Kamiyama, Yasuo
Kyoto City Hosp.
25–16 Shibatani–Cho
Takatsuki–Shi
Osaka, Japan 569

Kaplan, John E.
Dept. of Physiology
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Karlstad, Michael D.
Dept. of Anesthesiology
Univ. Tenn. Med. Ctr.
1924 Alcoa Hwy.
Knoxville, TN 37920

Kazarian, Kirk K.
Surgery Div.
Naval Med. Research Instit.
8901 Wisconsin Ave./Bldg. 17
Bethesda, MD 20814

Kelly, Kathleen M.***
S.I.C.U.
Hackensack Med. Ctr.
Hackensack, NJ 07661

Kerstein, Morris D.
Dept. of Surgery
Hahnemann Univ.
Broad & Vine Sts.
Philadelphia, PA 19102

Kilpatrick, Laurie
Div. of Immun. and Inf. Diseases
Children's Hospital of Phila.
34th St. and Civic Center Blvd.
Philadelphia, PA 19104

Kline, Mark W.
Dept. of Pediatrics
St. Louis Univ.
1465 S. Grand Blvd.
St. Louis, MO 63104

Kober, Philip M.
2009 Harrison St.
Evanston, IL 60201

Kovach, Aristztid G.B.
Cerebrovascular Res. Ctr., Rm. 429
Univ. of Penn., Johnson Pavillion
36th and Hamilton Walk
Philadelphia, PA 19104

Koyama, Shozo
Dept. of Physiology
Shinshu Univ. Sch. Med.
3-1-1 Ashai, Matsumoto
Nagano, Japan 390

Kramer, George C.
Dept. of Human Physiology
Sch. Med.
Univ. California, Davis
Davis, CA 95616

Krausz, Michael M.
Dept. of Surgery B
Hadassah Univ. Hosp.
Jerusalem, Israel 91120

Kreis, David J.
Chief of Trauma
Health Sci. Ctr. (T19,020)
SUNY-Stony Brook
Stony Brook, NY 11794

Kudsk, Kenneth A.
Surgery/Rm. E228, Coleman Bldg.
Univ. Physicians Fndn., Inc.
956 Court Ave.
Memphis, TN 38163

Kunkel, Steven L.
Dept. of Pathology
Univ. Michigan
1301 Catherine Rd.
Ann Arbor, MI 48109

Kutsky, Phyllis B.
4615 Edgefield Rd.
Bethesda, MD 20814

Lamonica Groves, Concetta R.
Admitting Area/MIEMSS
Univ. Maryland
22 S. Greene St.
Baltimore, MD 21201

Lang, Charles
Dept. of Physiology
LSUMC
1901 Perdido St.
New Orleans, LA 70112

Langdale, Lorrie A.
Surgical Service (112)
V.A. Med. Ctr.
1660 S. Columbian Way
Seattle, WA 98108

Lanza-Jacoby, Susan
Dept. of Surgery
Jefferson Med. Col.
1025 Locust St.
Philadelphia, PA 19107

Law, William R.
Naval Med. Res. Inst.
National Naval Med. Ctr.
Bldg. 21, Rm. 206, Mail Stop 15
Bethesda, MD 20814

Lechner, Robert
Dept. of Anesthesiology
Univ. Virginia
Health Sci. Ctr., Box 238
Charlottesville, VA 22908

Ledgerwood, Anna M.
Detroit Receiving Hosp.
Univ. Health Ctr.
4201 St. Antoine
Detroit, MI 48201

Lee, Bing C.
214 Lancaster Dr.
Piscataway, NJ 07054

Lee, Patrice A.
Humana Hosp. Med. City–Dallas
7777 Forest Lane
Suite c–740
Dallas, TX 75230

Lefer, Allan M.
Dept. of Physiology
Jefferson Med. Col.
1020 Locust St.
Philadelphia, PA 19107

Levenson, Stanley M.
Yeshiva Univ.
Albert Einstein Col. Med.
1300 Morris Park/Rm. 740 Forch.
Bronx, NY 10461

Levy, Jerrold H.
Dept. of Anesthesiology
Emory Hosp.
1364 Clifton Rd. N.E.
Atlanta, GA 30322

Lewis, David H.
Grangatan 7
Linkoping, Sweden S-58245

Lewis, Frank R.
Dept. of Surgery, Ward 3A25
San Francisco Gen. Hosp.
1001 Potrero Ave.
San Francisco, CA 94110

Ligas, James R.
Dept. of Surgery
St. Francis Hosp. & Med. Ctr.
114 Woodland St.
Hartford, CT 06105

Little, Roderick A.
North Western Injury Res. Ctr.
Univ. Manchester
Stopford Bldg., Oxford Rd.
Manchester, UK MI39PT

Liu, Maw-Shung
Dept. of Physiology
St. Louis Univ. Sch. Med.
1402 S. Grand Blvd.
St. Louis, MO 63104

Livingston, David H.
Dept. of Surgery
UMD–New Jersey
Univ. Hospital C–384
Newark, NJ 07103

Lobe, Thom E.
Dept. of Surgery
956 Court Ave.
Suite G212
Memphis, TN 38163

Loegering, Daniel J.
Dept. of Physiology/A134
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Longnecker, David E.
Dept. of Anesthesiology
Univ. Virginia Med. Ctr.
P.O. Box 238
Charlottesville, VA 22908

Lorenz, Wilfried
Klinikum Der Philipps
Univ. Marburg
Zentrum fur Operative Med. I
3550 Marburg, Germany

Lucas, Charles E.
Dept. of Surgery
Wayne State Univ.
540 E. Canfield
Detroit, MI 48201

Luebbe, Andreas S.***
Apt. 230
Eichssportfeldstrasse 16
Berlin 19, Germany D-1000

Lund, Niels
Dept. of Anesthesiology
Univ. Rochester
601 Elmwood Ave.
Rochester, NY 14642

Lundberg, Dag
Dept. of Anesthesiology
University Hospital
S-221 85
Lund, Sweden

Lundsgaard-Hansen, P.
Dept. of Experimental Surgery
Inselspital
P.O. Box 10
Berne, Switzerland CH3010

Luo, Zheng Yao
Dept. of Pathophysiology
Hunan Med. Col.
Changsha Hunan
Hunan, China

Lust, Robert M.
Div. of Cardiac Surg.
Brody Bldg. 45–22
E. Carolina Med. Ctr.
Greenville, NC 27858

Lutherer, Lorenz O.
Dept. of Physiology
Texas Tech. Univ.
Health Science Ctr.
Lubbock, TX 79430

Lysz, Thomas W.
Dept. of Surgery
New Jersey Med. Sch.
185 South Orange Ave.
Newark, NJ 07103

Machiedo, George W.
Dept. of Surgery E–350
New Jersey Med. Sch.
100 Bergen St.
Newark, NJ 07103

Maier, Ronald V.
Dept. of Surgery ZA-16
Harborview Med. Ctr.
Univ. Washington
Seattle, WA 98104

Maitra, Subir
Dept. of Surgery
SUNY at Stony Brook
Health Sci. Ctr. T19060
Stony Brook, NY 11794

Maksad, Ali K.
91 Bayview Ave.
E. Providence, RI 02915

Malangoni, Mark A.
Dept. of Surgery
3395 Scranton Rd.
Cleveland, OH 44109

Malcolm, Diana S.
Dept. of Surgery
Uniformed Serv. Univ.
4301 Jones Bridge Rd.
Bethesda, MD 20889

Markov, Angel K.
2500 North State St.
Jackson, MS 39216

Marshall, Bryan E.
Dept. of Anesthesiology
Hosp. Univ. Pennsylvania
3400 Spruce St.
Philadelphia, PA 19104

Marshall, Carol
Dept. of Anesthesiology
Univ. Pennsylvania
3400 Spruce St.
Philadelphia, PA 19104

Marshall, Lawrence F.
Surg. Neurosurg./H893
Univ. Calif. San Diego Med. Ctr.
225 Dickinson St.
San Diego, CA 92103

Martin, Louis F.
Dept. of Surgery
Milton S. Hershey Med. Ctr.
Penn. State Univ./P.O. Box 850
Hershey, PA 17033

Martyn, Jeevendra
Dept. of Anesthesia
Massachusetts Gen. Hosp.
Boston, MA 02114

Marzella, Louis
Dept. of Pathology and MIEMSS
Univ. Maryland
10 S. Pine St.
Baltimore, MD 21201

Marzi, Ingo***
Dept. of Trauma-Surgery
Univ. Saarland
Hamburg/Saar, Germany D-6650

Massion, Walter H.*
4700 Willard Ave.
Oklahoma City, OK 73105

Matera, Giovanni
Via de Nava 4
89100 Reggio
Calabria, Italy

Matson, James R.**
Pediatric Critical Care
Humana Hosp.-Med. City
7777 Forest Ln.
Bldg. A, Floor 12
Dallas, TX 75230

Matuschak, George M.
Dept. of Pulmonary Disease
St. Louis Univ. Med. Ctr.
3635 Vista Ave. at Grand Blvd.
St. Louis, MO 63110

McArdle, A. Hope
Univ. Surgical Clinic
Montreal Gen. Hosp.
1650 Cedar Ave.
Montreal, PQ, Canada H3G1A4

McCallum, R.E.
Microbiology and Immunology
Univ. Oklahoma Health Sci. Ctr.
P.O. Box 26901
Oklahoma City, OK 73190

McConn, Rita
40 S. Broadway
Irvington-on-Hudson
New York, NY 10533

McCoy, Sue
Box 265
Mountain Home, TN 37684

McCuskey, Robert S.
Dept. of Anatomy
Univ. Arizona
Health Sci. Ctr.
Tucson, AZ 85724

McDonough, Kathleen H.
Dept. of Physiology
Louisiana State Univ. Med. Ctr.
1901 Perdido St.
New Orleans, LA 70112

McIntosh, Tracy K.
Dept. of Surgery
Univ. Connecticut Hlth. Ctr.
Surgery Res. Ctr., Rm. L-1096
Farmington, CT 06032

McKenna, Thomas M.
Casualty Care Res.
Naval Med. Res. Inst.
Metabolic-MS42
Bethesda, MD 20814

McMillen, Marvin A.**
Dept. of Surgery
West Haven VAMC
Wet Spring St.
West Haven, CT 06516

McNamara, J. Judson
Dept. of Surgery
Univ. Hawaii Sch. Med.
1301 Punchbowl St.
Honolulu, HI 96813

McSwain, Norman E.
Dept. of Surgery
Tulane Univ. Med. Ctr.
1430 Tulane Ave.
New Orleans, LA 70112

Mela-Riker, Leena M.*
Dept. of Surgery
Oregon Hlth. Sci. Univ.
Sch. Med.
Portland, OR 97201

Meldrum, Daniel***
3810 Rowland #5
Grand Rapids, MI 49546

Meng, Xian Jun
Inst. Basic Med. Sci. Res.
Gen. Hosp. PLA
28 Fu Xing Rd.
Beijing, China 100853

Meredith, Jay W.
Dept. of Surgery
Bowman Gray Sch. Med.
300 S. Hawthorne Rd.
Winston-Salem, NC 27103

Mihm, Frederick
Dept. of Anesthesia
Stanford Univ. Sch. Med.
Room S 278
Stanford, CA 94305

Mileski, William***
Dept. of Surgery
Univ. Washington
325 Ninth St., ZA-16
Seattle, WA 98104

Militello, Philip
MIEMS
Univ. Maryland
22 S. Greene St.
Baltimore, MD 21201

Miller, Harvey I.
Dept. of Physiology
Louisiana State Med. Ctr.
1542 Tulane Ave.
New Orleans, LA 70112

Miller, Thomas A.
Dept. of Surgery
Univ. Texas Med. Sch.
6431 Fannin, MSB 4266
Houston, TX 77030

Moore, Ernest E.
Dept. of Surgery
Denver Gen. Hosp.
777 Bannock St.
Denver, CO 80204

Moore, James N.
Dept. Large Animal Med.
Col. Veterinary Med.
Univ. Georgia
Athens, GA 30602

Morgan, Anthony S.
St. Francis Hosp. and Med. Ctr.
114 Woodlawn St.
Hartford, CT 06105

Morita, Shigeho
Dept. of Anesthesia
Teikyo Univ.
3426-3 Anegasaki/Ichihara-City
Chiba, Japan 29901

Morris, Debra D.
Large Animal Med.
Univ. Georgia
Col. Vet. Med.
Athens, GA 30602

Morris, Jon B.
Dept. of Surgery
Univ. of Pennsylvania
4 Silverstein
Philadelphia, PA 19104

Moss, Gerald
Biomed. Engineering
Rensselaer Polytech. Inst.
Troy, NY 12181

Moss, Gerald S.
Office of the Dean (M/C 784)
Univ. Illinois Col. Med. West
Rm. 131, 1853 W. Polk St.
Chicago, IL 60612

Mouton, Wynand L.
P.O. Box 3974
Rustenburg, South Africa 0300

Mullins, Richard J.
Dept. of Surgery, L223A
Oregon Health Sci. Univ.
3181 S.W. Sam Jackson Park Rd.
Portland, OR 97201

Myers, Stuart I.**
Dept. of Gen. Surgery
U.T. Health Sci. Ctr.
6431 Fannin, MSB 4.162
Houston, TX 77030

Myrvold, Helge E.
Univ. Trondheim
Surgery/Regionsykehuset
I Trondheim
Trondheim, Norway 7000

Nagler, Arnold
Pre-Clinical Med. Educ.
N.Y. Col. Osteopathic Med.
P.O. Box 170 Wheatly
Old Westbury, NY 11568

Nance, Francis C.
Dept. of Surgery
St. Barnabas Med. Ctr.
Old Short Hills Rd.
Livingston, NJ 07039

Nauta, Russell J.
Dept. of Surgery
Georgetown Univ. Hosp.
3800 Reservoir Rd., N.W.
Washington, DC 20007

Neiberger, Richard E.
1302 N.W. 30 St.
Gainesville, FL 32605

Nelson, Karl M.
Dept. of Research
Baptist Med. Ctrs.–Princeton
701 Princeton Ave.
Birmingham, AL 35211

Nelson, Loren D.
218 Med. Ctr. South
2100 Pierce Ave.
Nashville, TN 37212

Nelson, William R.
Trauma Program
Sunnybrook Med. Ctr./Univ. Toronto
Room 4978, H Wing, 2075 Bayview
Toronto, Canada M4N 3M5

Nemoto, Edwin M.
Dept. of Anesthesiology
Univ. Pittsburgh
1081 Scaife Hall
Pittsburgh, PA 15261

Neugebauer, Edmund
II.ND Dept. of Surgery
Univ. Cologne
Ostmerheimer Strabe 200
Koln 91, Germany D-5000

Novelli, Gian Paolo
Policlinico Di Caregi
Inst. Anesthesiology and
Intensive Care
Florence, Italy 50134

O'Benar, John D.
Military Trauma Research
Letterman Army Inst. Rsch.
Presidio of San Francisco
San Francisco, CA 94129

Ochoa, Ricardo
Unit 7228-209-2
The Upjohn Co.
Portage, MI 49001

Oei, Howard
Neurosci./Cardiovasc. Res.
CIBA-GEIGY Pharmaceutical Div.
556 Morris Ave.
Summit, NJ 07901

Oestern, Hans-Jorg
Unfallchirurg Abteilung
Allgemeines Krankenhaus Celle
Siememsplatz 4
Celle, Germany 3100

Ogata, Hiromaru
Dept. of Anesthesiology
Dokkyo Univ. Sch. Med.
880 Mibu Shimotsuga Gun
Tochigi Pref, Japan 321-02

Ogawa, Ryo
Dept. of Anesthesiology
Nippon Med. Sch.
Sendagi 1-1-5, Bunkyo-ku
Tokyo, Japan 113

Ohkawa, Masanori
Matsuzawa Clinic
Kamiichiba Mutsuzawa
Chosei-Gun
Chiba 299-44, Japan

Ohtake, Yoshio
Emerg. and Crit. Care Med.
Chiba Univ. Sch. Med.
1-8-1 Inohana
Chiba, Japan

Okabe, Eiichiro
Pharmacology
Kanagawa Dental Col.
82-Inaokacho/238 Yokosuka
Kanagawa, Japan

Okada, Kazuo
Dept. of Anesthesiology
Teikyo Univ.
2-11-1 Kaga Itabashi-ku
Tokyo, Japan 173

Okuda, Minoru
Hatudai 1-49-3-301
Shibuya-Ku
Tokyo, Japan 151

Oldham, Keith
Sect. Pediatric Surgery
Room F7916/Mott Children's Hosp.
Univ. Michigan
Ann Arbor, MI 48109

Olson, Stephen E.**
Dept. of Gen. Surgery
Univ. Chicago
5841 S. Maryland Ave.
Chicago, IL 60637

Omann, Geneva M.
Dept. of Gen. Surg., Res. SVC151
Univ. Michigan
2215 Fuller Rd.
Ann Arbor, MI 48105

Osler, Turner
Dept. of Surgery
Univ. New Mexico
2211 Lomas Blvd.
Albuquerque, NM 87131

Panacek, Edward A.
Dept. of Critical Care
2074 Abington Rd.
Cleveland, OH 44106

Papadakos, Peter J.***
Surg. ICU, Dept. Anesthesiol.
Box 604
Univ. Rochester
601 Elmwood Ave.
Rochester, NY 14642

Parker, Janet L.
Dalton Research Ctr.
Research Park
Univ. Missouri
Columbia, MO 65211

Parratt, James R.
Dept. of Physiology & Pharmacology
Univ. Strathclyde Royal Col.
204 George St.
Glasgow, Scotland G1 1XW

Passmore, John C.
Physiology and Biophysics
Univ. Louisville Sch. Med.
Louisville, KY 40292

Pate, James W.
Dept. of Surgery
Univ. Tennessee
956 Court Ave.
Memphis, TN 38163

Patterson, C. Richard
Univ. Physicians Fndn.
956 Court Ave.
Suite G 218
Memphis, TN 38163

Paxson, Charles L.
5975 Carmen Ave.
Inver Grv. Hts., MN 55075

Pearce, Frederick J.
Dept. of Surgery
Univ. Rochester Sch. Med.
575 Elmwood Ave.
Rochester, NY 14642

Peitzman, Andrew P.
Dept. of Surgery
Univ. Pittsburgh Sch. Med.
1087 Sciafe Hall
Pittsburgh, PA 15261

Phillips, Robert W.
Physiology and Biophysics
Colorado State Univ.
Fort Collins, CO 80523

Pinsky, Michael R.
Dept. of Anesthesiology
Univ. Pittsburgh
3550 Terrace St.
Pittsburgh, PA 15261

Pivon, Richard J.
Experimental Surgery
McGill Univ.
740 Dr. Penfield Ave.
Montreal, Que., Canada H3A 1A4

Plemmons, Bob C.
Professional Communications
Upjohn International Inc.
7000 Portage Rd.
Kalamazoo, MI 49001

Pohlman, Timothy H.
Dept. of Surgery, RF-25
Univ. Washington
Seattle, WA 98192

Poole, Galen
Dept. of Surgery
Univ. Mississippi Med. Ctr.
2500 N. State St.
Jackson, MS 39216

Proctor, Herbert J.
Dept. of Surgery
Univ. North Carolina
at Chapel Hill
Chapel Hill, NC 27514

Pruitt, Basil A.
U.S. Army Inst. Surgical Research
Ft. Sam Houston, TX 78234

Pryor, Robert W.**
7777 Forest Lane
Bldg. A, Floor 12
Dallas, TX 75230

Puri, Vinod
Critical Care Services
Providence Hosp.
16001 W. Nine Mile Rd.
Southfield, MI 48075

Rackow, Eric C.
Dept. of Medicine
N.Y. Med. Coll.
St. Vincent's Hosp. & Med. Ctr.
153 W. 11th St.
New York, NY 10011

Rao, Papineni
Ob/Gyn Box 18
Univ. South Florida Col. Med.
12901 N. 30th St.
Tampa, FL 33612

Raymond, Richard M.
Cerebral Blood Flow Lab.
Loyola Univ. Med. Ctr.
2160 S. First Ave.
Maywood, IL 60153

Reed, R. Lawrence
Dept. of Surgery
U.T. Med. Sch.
L.B.J. General Hosp.
5656 Kelley St./Ste. 30S 62 008C
Houston, TX 77026

Reichard, Sherwood M.
Div. of Radiobiology
Med. Col. Georgia
1120 15th St.
Augusta, GA 30912

Reines, H. David
Med. Col. Virginia
Box 475, MCV Station
Richmond, VA 23298

Remick, Daniel
Dept. of Pathology
Univ. Michigan
Box 0602, 1301 Catherine Rd.
Ann Arbor, MI 48109

Renzi, Paula M.***
Physiology
Thomas Jefferson Univ.
1020 Locust St., Rm. 418 JAH
Philadelphia, PA 19107

Reynolds, David G.
Dept. of Surgery
Univ. South Florida
Tampa, FL 33612

Rhodes, Robert S.
Dept. of Surgery
Univ. Mississippi Sch. Med.
2500 N. State St.
Jackson, MS 39216

Rice, Charles L.
Dept. of Surgery
Harborview Med. Ctr.
325 Ninth St., ZA-16
Seattle, WA 98104

Rickert, William B.*
224 Yam Gandy Rd.
Savannah, GA 31411

Rink, Richard D.
Dept. of Anatomy
Univ. Louisville Health Sci. Ctr.
Louisville, KY 40292

Risberg, Bo I.
Dept. of Surgery I
Univ. Goteborg
Sahlgren's Hosp.
Goteborg, Sweden S-41345

Rocha-e-Silva, Mauricio
Research Div.
Instituto do Coracao
Av. Eneas Carvalhd Aguiar 44
São Paulo, Brazil 05403

Rodning, Charles B.
2451 Fillingim St.
Mobile, AL 36617

Rodriguez, Jorge L.
Dept. of Surgery
Univ. Michigan
1500 E. Med. Ctr. Dr.
Ann Arbor, MI 48109

Rogers, Charles E.
Dept. of Surgery
St. Francis Hosp.
100 Port Washington Blvd.
Roslyn, NY 11576

Rogers, Frederick**
Dept. of Surgery
Univ. Vermont
Given Bldg., Room D–319
Burlington, VT 05405

Rose, Stefan**
Trauma Surgery
Univ. of Saarland
Med. Ctr.
Homburg–Saar, Germany 6650

Rosen, Arthur L.
2323 Schiller Ave.
Wilmette, IL 60091

Rowe, Marc I.
Dept. of Surgery
Children's Hosp.
3705 Fifth Ave. at Desoto
Pittsburgh, PA 15213

Rush, Benjamin F.
Dept. of Surgery
New Jersey Med. Sch.
185 S. Orange Ave./Rm. G506
Newark, NJ 07103

Saba, Thomas M.
Dept. of Physiology/MS. 341
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Sacco-Gibson, Nancy A.**
Anatomy/Medical
Loyola Univ. Chicago Med. Sch.
2160 S. First Ave.
Maywood, IL 60153

Safar, Peter
Resuscitation Research Ctr.
Univ. Pittsburgh
3434 Fifth Ave.
Pittsburgh, PA 15260

Samuels, Sharon B.**
Dept. of Surgery
Univ. Connecticut
263 Farmington Ave.
Farmington, CT 06032

Sanan, Saroj**
210 Basant Ave.
Amritsar
Punjab, India 143001

Sayeed, Mohammed M.
Dept. of Physiology
Loyola Univ. Stritch Sch. Med.
2160 S. First Ave.
Maywood, IL 60153

Scalea, Thomas M.
Dept. of Surgery
SUNY-Health Sci. Ctr.
450 Clarkson Ave.
Brooklyn, NY 11203

Schaefer, Carl F.
Dept. of Anesthesiology
Univ. Oklahoma Health Sci. Ctr.
P.O. Box 26901, Research Bldg., 25R
Oklahoma City, OK 73190

Schertel, Eric R.
Veterinary Clinical Science
Ohio State Univ.
1935 Coffey Rd.
Columbus, OH 43210

Schlag, Gunther
FA. F Anesthesiologie
Cobenzglasse 68
Vienna, Austria/Europe A-1190

Schloerb, Paul*
Dept. of Surgery
Univ. Kansas Med. Ctr.
Kansas City, KS 66103

Schmahl, Frederich W.
Inst. fur Arbeits und Sozialmedizin
Wilhelmstr 27
Tuebingen, Germany 7400

Scholten, Donald J.**
Dept. of Surgery
Michigan State Univ.
100 Michigan Ave., N.E.
Grand Rapids, MI 49503

Schumer, William
Dept. of Surgery
Univ. Health Sci.
Chicago Med. Sch.
North Chicago, IL 60064

Sehgal, Lakshman R.
Northfield Laboratories
Suite 200
1200 N. Business Ctr. Dr.
Mt. Prospect, IL 60056

Selkurt, Ewald E.
12999 N. Penn Ave.
Carmel, IN 46032

Semrad, Susan
Med. Sci. Large Animal
Univ. Wisconsin-Madison
Sch. Vet. Med., 2015 Linden Dr. W.
Madison, WI 53706

Serkes, Kenneth D.
860 Ladera Lane
Montecito, CA 93108

Shackford, Steven R.
Dept. of Surgery
Univ. Vermont
Fletcher House 301, MCHV
Burlington, VT 05401

Shah, Dhiraj M.
Dept. of Surgery/ME 602
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Shaikh, Khaleel A.
15 Wyntre Brooke Dr.
York, PA 17403

Shapiro, Marc J.
Dept. of Surgery
St. Louis Univ.
3635 Vista, P.O. Box 15250
St. Louis, MO 63110

Shatney, Clayton
Dept. of Surgery
Santa Clara Valley Med. Ctr.
751 S. Bascom Ave.
San Jose, CA 95128

Shenep, Jerry
Dept. of Infectious Disease
St. Jude Children's Hosp.
332 N. Lauderdale
P.O. Box 318
Memphis, TN 38101

Shennib, Hani
Dept. of Surgery
Montreal Gen. Hosp.
1650 Cedar St.
Montreal, Quebec, Canada H3G 1A4

Shepherd, Raymond E.
Dept. of Physiology
LSU Med. Ctr.
1901 Perdido St.
New Orleans, LA 70112

Shigematsu, Hiroshi
1st Dept. of Surgery
Fac. Med. Univ. Tokyo
7-3-1 Hongo Bunkyo-ku
Tokyo, Japan 113

Shiono, Shigeru***
Dept. of Traumatology Emer. Unit
Matsudo Municipal Hosp.
Kamihongoh 4005
Matsudo City, Japan 271

Shires, George Thomas, III
Dept. of Surgery
Univ. TX Southwestern Med. Ctr.
5232 Harry Hines Blvd.
Dallas, TX 75235

Short, Billie Lou
Neonatal Intensive Care
Children's Hosp. Med. Ctr.
111 Michigan Ave., N.W.
Washington, DC 20010

Sibbald, William J.
Dept. of Medicine
Victoria Hosp.
391 South St.
London, Ont, Canada N6A 465

Siegel, John H.
Deputy Director, MIEMSS
Univ. Maryland
22 S. Greene St.
Baltimore, MD 21201

Simms, H. Hank**
Dept. of Surgery, APC 144
Rhode Island Hosp.
593 Eddy St.
Providence, RI 02903

Slater, Harvey
Mellon Pavilion
4815 Liberty Ave.
Pittsburgh, PA 15224

Slotman, Gus Jay
Dept. of Surgery
UMDNJ/Robert W. Johnson Med. Sch.
3 Cooper Plaza, Suite 411
Camden, NJ 08103

Smith, J. Stanley
Dept. of Surgery
M.S. Hershey Med. Ctr.
P.O. Box 850
Hershey, PA 17033

Smith, Edward F., III
Dept. of Pharmacology
Smith Kline & French Labs.
P.O.B. 1539 (L-510)
King of Prussia, PA 19406

Solomkin, Joseph S.
Dept. of Surgery
Univ. Cincinnati Col. Med.
231 Bethesda Ave.
Cincinnati, OH 45267

Spath, James A.
Dept. of Physiology
Thomas Jefferson Univ.
1020 Locust St.
Philadelphia, PA 19107

Spence, Richard K.
3 Cooper Plaza
Suite 411
Camden, NJ 08103

Spillert, Charles R.
Surgery/Med. Sci. Bldg. G505
UMDNJ–New Jersey Med. Sch.
185 S. Orange Ave.
Newark, NJ 07103

Spitzer, John J.
Dept. of Physiology
Louisiana State Med. Ctr.
1901 Perdido St.
New Orleans, LA 70112

Spitzer, Judy A.
Dept. of Physiology & Med.
Louisiana State Med. Ctr.
1901 Perdido St.
New Orleans, LA 70112

Spolarics, Zoltan
Dept. of Physiology
LSU Med. Ctr.
1901 Perdido St.
New Orleans, LA 70112

Sprung, Charles L.
Section of Critical Care Med.
VA Med. Ctr. (111)
1201 N.W. 16th St.
Miami, FL 33125

Stanford, Gregory
Dept. of Surgery
U.T. Southwestern Med. Sch.
5323 Harry Hines Blvd.
Dallas, TX 75235

Stein, Marshall D.
Shriners Burns Inst.
610 Texas Ave.
Galveston, TX 77550

Steingrub, Jay S.
15 McIntosh Dr.
Wilbraham, MA 01095

Stephan, Rabie N.
115 Mona Dr.
Amherst, NY 14226

Stewart, Kerry D.**
Dept. of CV Biology
Okla. Med. Res. Foun.
825 N.E. 13th St.
Oklahoma City, OK 73104

Stidham, Gregory L.
Univ. Physicians Fndn.
Dept. Critical Care, Lebonheur
One Children's Plaza
Memphis, TN 38103

Stith, Rex D.
Dept. of Physiology
Univ. Oklahoma Hlth. Sci. Ctr.
P.O. Box 26901
Oklahoma City, OK 73190

Stothert, Joseph C., Jr.
Dept. of Surgery, E 45
Univ. Texas Med. Branch
301 Univ. Blvd.
Galveston, TX 77550

Straughn, Fred K.**
7777 Forest Lane
Bldg. A, Floor 12
Dallas, TX 75230

Stremple, John
Dept. of Surgery
V.A. Hosp.
University Dr. C
Pittsburgh, PA 15240

Su, Jing-Yi
Dept. of Pathophysiology
Beijing Med. Univ.
Xue Yuan Rd.
Beijing, China

Sugerman, Harvey J.
Dept. of Surgery
Med. Col. Virginia
Box 519
Richmond, VA 23229

Sumpio, Bauer E.
Dept. of Surgery
Yale Univ. Sch. Med.
333 Cedar St.
New Haven, CT 06510

Talucci, Raymond C.
Dept. of Surgery
Three Cooper Plaza
Suite 411
Camden, NJ 08103

Tang, Chaoshu
Dept. of Pathophysiology
Beijing Med. Univ.
Xue Yuan Rd.
Beijing, China

Taylor, Fletcher B.
Thrombosis/Hematol. Res. Progr.
Oklahoma Med. Research Fndn.
825 N.E. 13th St.
Oklahoma City, OK 73104

Taylor, Glen A.
Dept. of Surgery
Sunnybrook Med. Ctr.
2075 Bayview Ave.
Toronto, Ont, Canada M4N 3M5

Teba, Luis
Dept. Pulmonary Cnt. Care Med.
W.V.U. Health Science Ctr.
Morgantown, WV 26506

Teller, John D.
Cardiovascular Disease
The Upjohn Company
RD #4, Box 445/Turf Dr.
Freehold, NJ 07728

Tempel, George E.
Dept. of Physiology
Med. Univ. South Carolina
171 Ashley Ave.
Charleston, SC 29425

Thijs, Lambertus G.
Med. Intensive Care Unit
Free Univ. Hosp.
De Boelelaan 1117, 1081 HV
Amsterdam, Netherlands

Thomson, Stewart J.S.
34 Deane Crescent
Northmead Ext 7
Benoni 1500, South Africa

Till, Gerd O.
Dept. of Pathology
Univ. Michigan Med. Sch.
1301 Catherine Rd.
Ann Arbor, MI 48109

Timberlake, Gregory A.
Dept. of Surgery
West Virginia Univ.
Health Sci. Ctr. North
Morgantown, WV 26506

Todd, Thomas R.J.
Toronto Gen. Hosp.
101 College St./10 EN
Toronto, Ontario, Canada M5G 1L7

Toledo-Pereyra, Luis H.
Dept. Surgery
Mount Carmel Mercy Hosp.
6071 W. Outer Dr.
Detroit, MI 48235

Tompkins, Ronald G.
Dept. of Surgery
Mass. Gen. Hosp.
ACC4 Suite 464
Boston, MA 02114

Torma, Michael J.
Med. Ctr. Scott/SG
Scott AFB, IL 62225

Toth, Phillip D.
Midwest Res. Inst., Inc.
3266 N. Meridian St.
Suite 203
Indianapolis, IN 46208

Townsend, Michael C.
Dept. of Surgery
Ohio State Univ.
410 W. 10th Ave., N–723 Doan Hall
Columbus, OH 43210

Traber, Daniel L.
Anesthesia Research
Shriners Burns Inst.
610 Texas Ave.
Galveston, TX 77550

Traber, Lillian D.***
Dept. of Anesthesiology
Univ. Texas Med. Branch
610 Texas Ave.
Galveston, TX 77550

Tracey, Kevin J.
Dept. of Neurosurgery
N.Y. Hosp./Cornell Univ. Med. Ctr.
525 E. 68th St.
New York, NY 10021

Treat, Richard C.
Dept. of Surgery
Univ. Alabama
U.A.B. Station
Birmingham, AL 35294

Trentz, Otmar L.
Dept. of Surgery–Trauma
Univ. Saarland
Chirurgische Univ. Klinik
Homburg S., Germany 665

Troop, Bryan
621 S. New Ballas, 1017
St. Louis, MO 63141

Trooskin, Stanley Z.
Dept. of Surgery
SUNY Health Sci. Ctr., Bklyn
450 Clarkson Ave., Box 40
Brooklyn, NY 11203

Trump, Benjamin F.
Dept. of Pathology
Univ. Maryland
10 S. Pine St.
Baltimore, MD 21201

Trunkey, Donald
Dept. of Surgery
Oregon Health Sci. Univ.
3181 S.W. Sam Jackson Park Rd.
Portland, OR 97201

Turinsky, Jiri
Dept. of Physiology
Albany Med. Col.
47 New Scotland Ave.
Albany, NY 12208

Tyler, Jeff W.**
Large Animal Surgery and Med.
College Vet. Med.
Auburn Univ.
Auburn, AL 36849

Ulevitch, Richard J.
Dept. of Immunopathology
Scripps Clinic & Research Fndn.
10666 N. Torrey Pines Rd.
La Jolla, CA 92037

Unger, Lauren S.**
Physiology and Biophysics
Univ. Louisville
A1110 Health Sci. Ctr.
Louisville, KY 40292

Urbaschek, Bernhard
Trubnerstrabe 38
Heidelberg, Germany 6900

Urbaschek, Renate
Inst. Med. Mikrobiol. & Hygiene
Klinikum Mannheim
Theodor-Kutzer-Ufer
Mânnheim 1, Germany 6800

Van Der Meer, Cornelis*
Dept. of Pharmacology
Univ. Amsterdam
Polderweg 104
Amsterdam, Netherlands 1093 KP

Van Kesteren, R.G.
Univ. Hosp.
Dept. Reanimation and
Clinical Toxicology
Utrecht, Netherlands 3500 CQ

Vargish, Thomas
Dept. of Surgery
Univ. Chicago
5841 S. Maryland Ave.
Chicago, IL 60637

Vary, Thomas C.
Dept. of Physiology
Milton S. Hershey Sch. Med.
Pennsylvania State Univ.
Hershey, PA 17033

Velasco, Irineu
Res. Div.
The Heart Inst.
Caixa Postal 11450
Sao Paulo, SP
Brazil 05499

Vincent, Jean-Louis
Dept. of Intensive Care
Erasme Univ. Hosp.
Route De Lennick 808
Brussels, Belgium 1070

Voeller, Guy R.
Dept. of Surgery
Univ. Tennessee
956 Court Suite G218
Memphis, TN 38163

Wade, Charles E.
Military Trauma
Letterman Army Inst.
SGRD-UL-MT
San Francisco, CA 94129

Waeber, Bernard
Dept. of Hypertension
Univ. Hosp. (CHUV)
Lausanne, Switzerland 1011

Wang, Ping
Dept. of Surgery
Mich. State Univ.
B424 Clinical Ctr.
East Lansing, MI 48824

Watkins, W. David
Dept. of Anesthesiology
P.O. Box 3094
Duke Univ. Med. Ctr.
Durham, NC 27710

Weil, Max
Univ. Health Sci.
Chicago Med. Sch.
North Chicago, IL 60064

Weireter, Leonard**
Dept. of Surgery
Eastern Virginia Med. Sch.
825 Fairfax Ave.
Norfolk, VA 23507

Welch, Gary W.
Dept. of Anesthesia
Univ. Massachusetts
55 Lake Ave. N.
Worcester, MA 01655

Wertz, E. Jean**
Pediatric ICU
Medical City
7777 Forest Lane
Dallas, TX 75230

West, Michael A.
Dept. of Surgery
Hennepin County Med. Ctr.
701 Park Ave.
Minneapolis, MN 55415

Whidden, Stanley John
Baromed. Research Inst.
JESMC
2917 Pyrtania
New Orleans, LA 70115

Williams, Lester F.
Professor of Surgery
Vanderbilt Univ. Sch. of Med.
4220 Harding Rd., Box 380
Nashville, TN 37202

Wilmoth, Frank R.
1803 N. Club Ct.
Tampa, FL 33612

Wilson, Michael F.
Dept. of Med. & Radiobiology
V.A. Med. Ctr. (151)
921 N.E. 13th St.
Oklahoma City, OK 73104

Wilson, Robert F.
Dept. of Surgery
Wayne State Univ.
6C-UHC
4201 St. Antoine
Detroit, MI 48201

Winn, Robert K.
Dept. of Surgery ZA-16
325 9th Ave.
Seattle, WA 98104

Wise, W. Curtis
Dept. of Physiology
Med. Univ. South Carolina
171 Ashley Ave.
Charleston, SC 29425

Wisner, David H.
Dept. of Surgery
UCD Med. Ctr.
4301 X St., Rm. 2310
Sacramento, CA 95817

Witek-Janusek, Linda
Dept. of Physiology
Loyola Univ. Med. Ctr.
2160 First Ave.
Maywood, IL 60153

Wolf, Matthew B.
Dept. of Physiology
Univ. South Carolina
Sch. Med.
Columbia, SC 29208

Wolfe, Robert R.
Metabolism Unit
Shriners Burns Inst.
610 Texas Ave.
Galveston, TX 77550

Wu, Chih-Hsiung
Dept. of Surgery
Taipei Med. Col.
252 Wu-Hsing Street
Taipei, Taiwan (ROC)

Yamamoto, Yasuhiro
Nippon Med. Sch.
1-1-5 Sendagi
Bunkyo-ku
Tokyo, Japan

Yelich, Michael R.
Dept. of Physiology
17 Elizabeth Ave.
Downersgrove, IL 60516

Yeston, Neil S.
Hartford Hosp.
80 Seymour St.
Hartford, CT 06115

Young, Jamie S.***
Dept. of Physiology, Sch. Med.
Univ. Virginia
Box 1116, MR4–Annex
Charlottesville, VA 22908

Young, Wise
Dept. of Neurosurgery
N.Y.U. Med. Ctr.
550 First Ave.
New York, NY 10016

Yu, Thomas L.***
Boston Univ. Hosp.
Dept. of Laboratory Med.
88 E. Newton St.
Boston, MA 02118

Yuan, Xiao Q.
Military Trauma Re.
Letterman Army Inst.
Presidio
San Francisco, CA 94129

Zaloga, Gary P.
Anesthesia/Crit. Care Med.
Bowman Gray Sch. Med.
300 S. Hawthorne Rd.
Winston-Salem, NC 27103

Zapol, Warren M.
Dept. of Anesthesia
Massachusetts Gen. Hosp.
Fruit St.
Boston, MA 02114

Zdon, Michael
V.A. Med. Ctr.
Surgical Service 112
3001 Green Bay Rd.
North Chicago, IL 60064

Zeller, W. Patrick
Pediatr. Endocr. Metabol. & Nutr.
Loyola Univ. Med. Ctr.
2160 S. First Ave.
Maywood, IL 60153

Zimmerman, Jerry J.
Dept. of Pediatrics
Univ. Wisconsin Child Hosp.
H4/470 CSC, 600 Highland Ave.
Madison, WI 53792

LISTING BY STATE

ALABAMA
Brinson, Robert R.
Cain, Stephen M.
Dyess, Donna Lynn
Fletcher, John Raymond
Glenn, Thomas M.
Nelson, Karl M.
Rodning, Charles B.
Treat, Richard C.
Tyler, Jeff W.

ARIZONA
Applefeld, Jack J.
Bajo, Thomas
Carmona, Richard
McCuskey, Robert S.

ARKANSAS
Cone, John B.

CALIFORNIA
Abraham, Edward
Arfors, Karl E.
Blaisdell, F. William
Carroll, Gilbert C.
Cryer, H. Gill
Emerson, Thomas E.
Gao, Guangcheng
Green, Douglas R.
Gunther, Robert A.
Hansbrough, John F.
Holcroft, James W.
Hoyt, David B.
Kramer, George C.
Lewis, Frank R.
Marshall, Lawrence F.
Mihm, Frederick
O'Benar, John D.
Serkes, Kenneth D.
Slatney, Clayton
Ulevitch, Richard J.
Wade, Charles E.
Wisner, David H.
Yuan, Xiao Q.

COLORADO
Fettman, Martin J.
Moore, Ernest E.
Phillips, Robert W.

CONNECTICUT
Casey, Kenneth F.
Cohn, Stephen M.
Hirvela, Elsa R.
Iba, Toshiaki
Ligas, James R.
McIntosh, Tracy K.
McMillen, Marvin A.
Morgan, Anthony S.
Samuels, Sharon B.
Sumpio, Bauer E.
Yeston, Neil S.

DELAWARE
Baker, Robert J.
Fagraeus, Lennart

DISTRICT OF COLUMBIA
Geelhoed, Glenn W.
Harmon, John W.
Nauta, Russell J.
Short, Billie Lou

FLORIDA
Baker, Carleton H.
Balis, John U.
Cane, Roy D.
Cavanagh, Denis
Flynn, Timothy C.
Neiberger, Richard E.
Rao, Papineni
Reynolds, David G.
Sprung, Charles L.
Wilmoth, Frank R.

GEORGIA
Hall, Edward L.
Levy, Jerrold H.
Moore, James N.
Morris, Debra D.
Reichard, Sherwood M.
Rickert, William B.

HAWAII
McNamara, J. Judson

IOWA
Ephgrave, Kimberly S.
Gurll, Nelson J.

ILLINOIS
Allen, Elizabeth J.
Alverdy, John C.
Barrett, John A.
Burhop, Kenneth E.
Dries, David J.
Ferguson, James L.
Fildes, John
Filkins, James P.
Gamelli, Richard L.
Goto, Masakatsu
Gould, Steven A.
Griffin, Andrew J.
Hall, John R.
Hirsch, Leroy J.
Hurley, R. Morrison
Jones, Stephen
Kober, Philip M.
Moss, Gerald S.
Olson, Stephen E.
Raymond, Richard M.
Rosen, Arthur L.
Sacco-Gibson, Nancy A.
Sayeed, Mohammed M.
Schumer, William
Sehgal, Lakshman R.
Torma, Michael J.
Vargish, Thomas
Weil, Max
Witek-Janusek, Linda
Yelich, Michael R.
Zdon, Michael
Zeller, W. Patrick

INDIANA
Babbs, Charles F.
Bottoms, Gerald D.
Cronen, Paul
Fessler, John F.
Griffin, David W.
Herron, David K.
Johnson, Martha A.
Selkurt, Ewald E.
Toth, Phillip D.
Schloerb, Paul

KANSAS
Asher, Eleanor, F.

KENTUCKY
Garrison, Richard N.
Groff, Diller B.
Harris, Patrick D.
Passmore, John C.
Rink, Richard D.
Unger, Lauren S.

LOUISIANA
Bagby, Gregory J.
Barker, Louis A.
Bowen, John C.
Conrad, Steven A.
Deitch, Edwin
Flint, Lewis M.
Lang, Charles
McDonough, Kathleen H.
McSwain, Norman E.
Miller, Harvey I.
Shepherd, Raymond E.
Spitzer, John J.
Spitzer, Judy A.
Spolarics, Zoltan
Whidden, Stanley John

MARYLAND
Allo, Maria D.
Breslow, Michael J.
Buchman, Timothy G.
Bulkley, Gregory B.
Chernow, Bart
Clemens, Mark G.
Cowley, R. Adams
Dunham, C. Michael
Gann, Donald S.
Hinson, Douglas
Kazarian, Kirk K.
Kutsky, Phyllis B.
Lamonica Groves, Concetta R.
Law, William R.
Malcolm, Diana S.
Marzella, Louis
McKenna, Thomas M.
Militello, Philip
Siegel, John H.
Trump, Benjamin F.

MASSACHUSETTS
Borlase, Bradley C.
Botan, Edward A.
Burke, John F.
Castillo, Leticia
Demling, Robert H.
Durkot, Michael John
Fiddian-Green, Richard
Fink, Mitchell P.
Grindlinger, Gene A.
Hechtman, Herbert B.
Martyn, Jeevendra
Steingrub, Jay S.
Tompkins, Ronald G.
Welch, Gary W.
Yu, Thomas L.
Zapol, Warren M.

MICHIGAN
Ayala, Alfred
Chaudry, Irshad H.
Cilley, Robert E.
Coran, Arnold G.
Dahn, Michael S.
Geiser, Ronald W.
Greenfield, Lazar J.
Guice, Karen S.
Harkema, James M.
Haupt, Marilyn T.
Hauptman, Joe
Hinshaw, Daniel B.
Jain, Krishna M.
Joyce, Harry H.
Kunkel, Steven L.
Ledgerwood, Anna M.
Lucas, Charles E.
Meldrum, Daniel
Ochoa, Ricardo
Oldham, Keith
Omann, Geneva M.
Plemmons, Bob C.
Puri, Vinod
Remick, Daniel
Rodriguez, Jorge L.
Scholten, Donald J.
Till, Gerd O.
Toledo-Pereyra, Luis H.
Wang, Ping
Wilson, Robert F.

MINNESOTA
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June 9–11, 1991

Eilat, Israel

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- The effect of shock resuscitation on the CNS
- Shock resuscitation in burns and smoke inhalation
- Oxygen therapy in shock resuscitation
- Blood substitutes in shock resuscitation
- Resuscitation of septic shock
- Hypertonic saline resuscitation in shock
- Multiple trauma resuscitation

FOR FURTHER INFORMATION, PLEASE CONTACT:

Michael M. Krausz, M.D., Chairman
Organizing Committee of the International Meeting on Shock Resuscitation
P.O.B. 50006
Tel Aviv 61500, Israel

CIRCULATORY SHOCK

Revised January 1991

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