The animal intensive care unit – how to design animal experiments in shock research

Peter Radermacher, Pierre Asfar

Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?

Angus & van der Poll:
Severe sepsis and septic shock.
NEJM 2013;369:840

"....Longer experiments with more advanced supportive care would allow better mimicry of the later stages of sepsis and multiorgan failure, permitting the testing of drugs in a more realistic setting...."

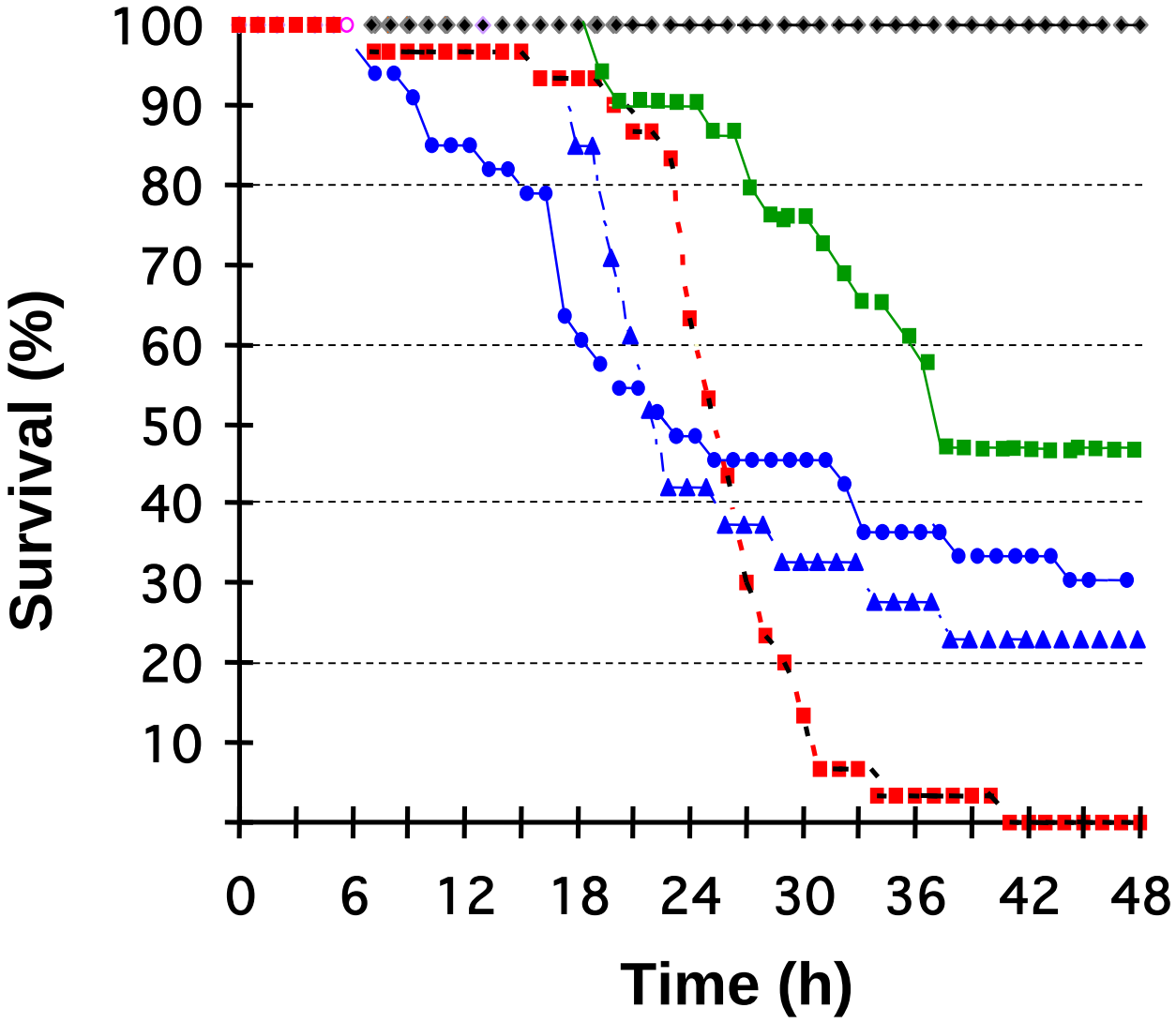
"....For example, options to enrich the preclinical portfolio include the study of animals that are more genetically diverse, are older, or have preexisting disease...."

Marini et al: **Critical care evidence – new directions.**
JAMA 2015;313:893

"....Development of appropriate experimental animal models or other preclinical research models should be considered a high priority of future research agendas...."

Traber DL : *Expired nitric oxide and shock in higher order species.* Crit Care Med 1999;27:255

"…Would you as a critical care physician accept data on a septic patient who was not resuscitated? Would you accept data from a drug study on an intensive care patient who was not only not resuscitated with fluid but who did not even have blood pressures and heart rates monitored?... If the animals are resuscitated, is the resuscitation to a specific physiologic variable?... The pathophysiology and outcome of an unresuscitated, unmonitored, septic patient is certainly different…."



Control group

Hollenberg SM et al: **Characterization of a hyperdynamic murine model of resuscitated sepsis using echocardiography.** ***AJRCCM 2001;164:891-5***

CLP+fluids +antibiotics

CLP+antibiotics

CLP+fluids

CLP no therapy

Do we need the „Animal ICU“?

Yes!

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?

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Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?

Mice as models for ALI/ARDS/shock

Pro:

- Genetically-modified strains available
- Homogeneus groups:
  - Incest strains -> little genetical variability
  - Gender selection possible
  - Young, healthy *or* specific co-morbidity, age
- Easy control of body temperature
- Numerous test systems available

Con:

- Size -> small blood volume, difficult instrumentation
- „non-shivering" thermogenesis
- different mediator release/response

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Genomic responses in mouse models poorly mimic human inflammatory diseases

Junhee Seok[a,1], H. Shaw Warren[b,1], Alex G. Cuenca[c,1], Michael N. Mindrinos[a], Henry V. Baker[c], Weihong Xu[a], Daniel R. Richards[d], Grace P. McDonald-Smith[e], Hong Gao[a], Laura Hennessy[f], Celeste C. Finnerty[g], Cecilia M. López[c], Shari Honari[f], Ernest E. Moore[h], Joseph P. Minei[i], Joseph Cuschieri[j], Paul E. Bankey[k], Jeffrey L. Johnson[h], Jason Sperry[l], Avery B. Nathens[m], Timothy R. Billiar[l], Michael A. West[n], Marc G. Jeschke[o], Matthew B. Klein[j], Richard L. Gamelli[p], Nicole S. Gibran[j], Bernard H. Brownstein[q], Carol Miller-Graziano[k], Steve E. Calvano[r], Philip H. Mason[e], J. Perren Cobb[s], Laurence G. Rahme[t], Stephen F. Lowry[r,2], Ronald V. Maier[j], Lyle L. Moldawer[c], David N. Herndon[g], Ronald W. Davis[a,3], Wenzhong Xiao[a,t,3], Ronald G. Tompkins[t,3], and the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program[4]

PNAS 2013;110:3507

Genomic responses in mouse models greatly mimic human inflammatory diseases

PNAS 2015;112:1167

Keizo Takao[a,b] and Tsuyoshi Miyakawa[a,b,c,1]

ABANDON THE MOUSE RESEARCH SHIP? NOT JUST YET!

Marcin F. Osuchowski[1,a], Daniel G. Remick[2], James A. Lederer[3], Charles H. Lang[4], Ansgar O. Aasen[5], Mayuki Aibiki[6], Luciano C. Azevedo[7], Soheyl Bahrami[1], Mihaly Boros[8], Robert Cooney[9], Salvatore Cuzzocrea[10], Yong Jiang[11], Wolfgang G. Junger[12], Hiroyuki Hirasawa[13], Richard S. Hotchkiss[14], Xiang-An Li[15], Peter Radermacher[16], Heinz Redl[1], Reinaldo Salomao[17], Amin Soebandrio[18], Christoph Thiemermann[19], Jean-Louis Vincent[20], Peter Ward[21], Yong-Ming Yao[22], Huang-Ping Yu[23], Basilia Zingarelli[24], and Irshad H. Chaudry[25,a]

Shock 2014;41:463

LPS doses

Human volunteers:

- **4 ng/kg bolus (Preas et al, AJRCCM 2001; 164:620)**
- **2 ng/kg bolus (Michaeli et al, Clin Nutr 2007;26:70)**

Mice, endotoxin-induced acute lung injury:

- **0.4 mg/kg bolus nasal (Dreymüller et al, EMBO Mol Med 2012;4:412)**
- **1.5 mg/kg bolus i.v. (Mangalmurti et al, Blood 2009;113:1158)**
- **4 mg/kg bolus intratracheal (Mekontso-Dessap et al, AJRCBM 2012;46:541)**

Pigs, endotoxin-induced „septic“ shock:

- **10-40 ng/kgxmin infusion (Hauser et al, CCM 2005;33:2034)**

NO metabolites

Human septic shock:

- **77 ± 10 µM (controls: 29 ± 4; non-septic trauma 13 ± 2 µM) (Ochoa et al, Ann Surg 1991;32:13)**
- **47 (28-77) µM (Watson et al, CCM 2004;32:13)**

Mice, CLP-induced septic acute lung injury:

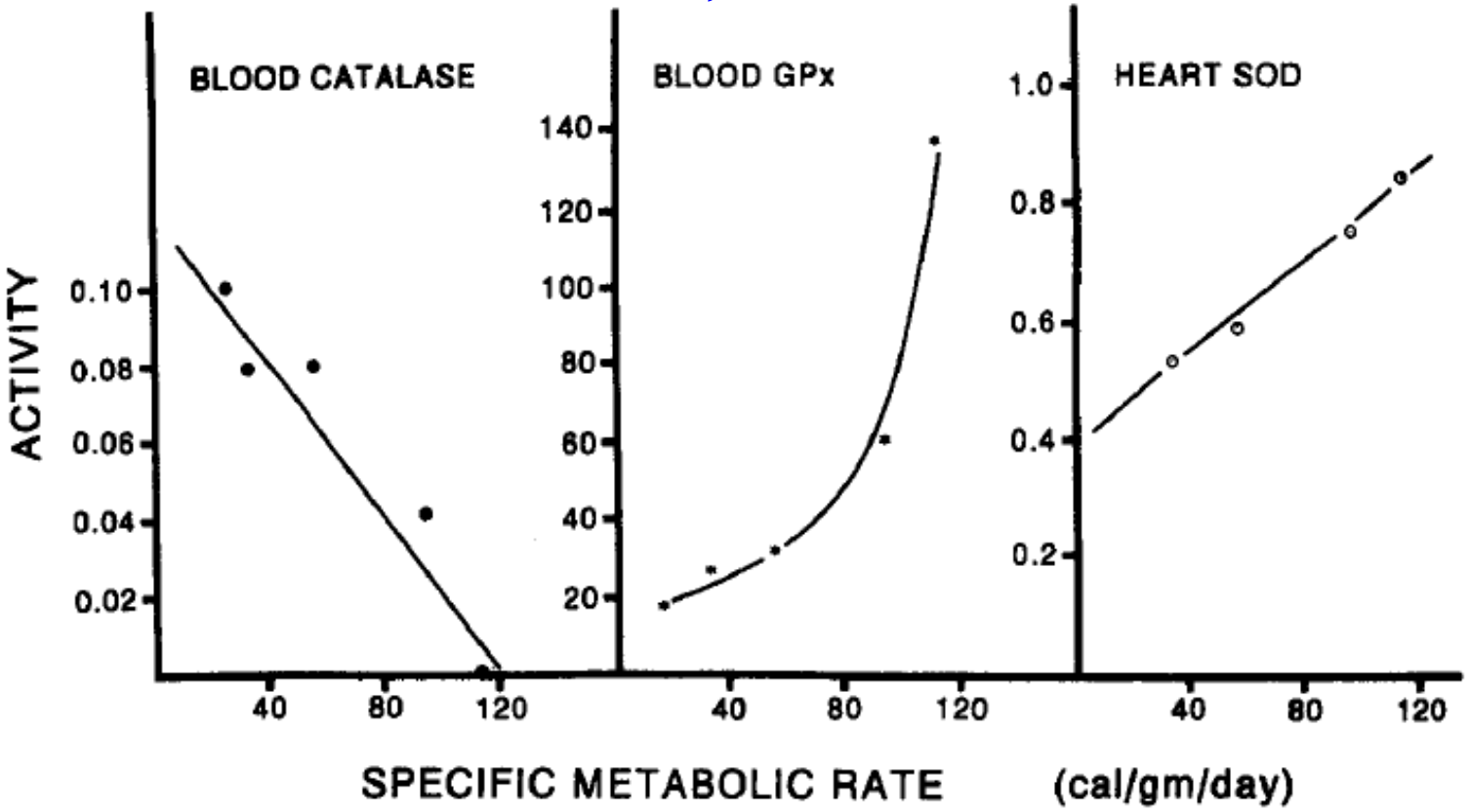
- **102 (47-121) µM (Barth et al, CCM 2006;34:307)**
- **161 ± 23 µM (Wang et al, Am J Pathol 2012;180:505)**

Pigs, fecal peritonitis-induced septic shock:

- **17 (11-30) µM (before shock: 21 (12-48) µM) (Simon et al, Crit Care 2009;13:R113)**

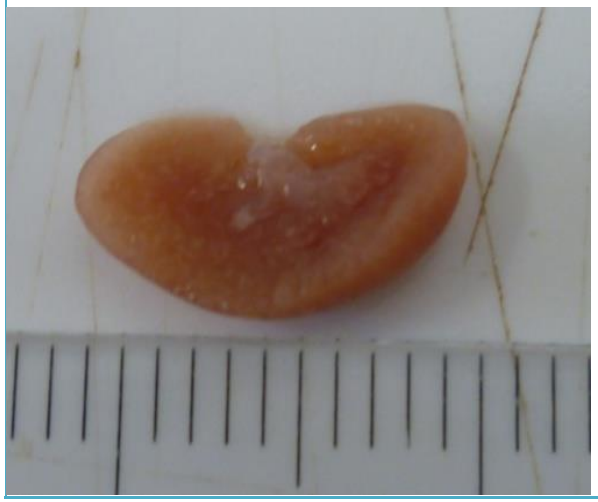
Godin DV, Garnett ME:

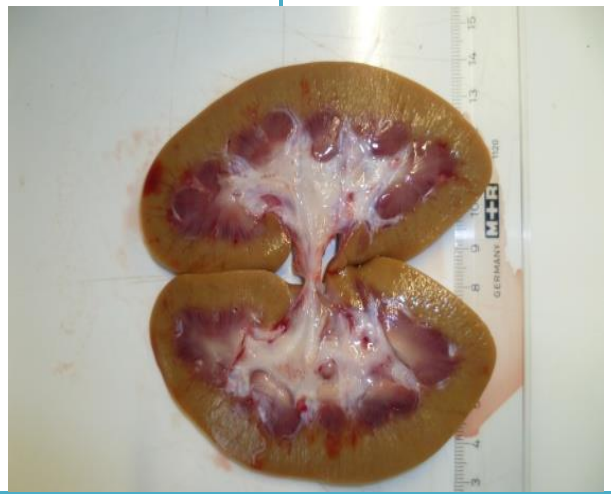
Species-related variations in tissue antioxidant status – II. Differenes in susceptibility to oxidative stress. ***Comp Biochem Physiol B 1992;103:743;*** **Species-related variations in tissue antioxidant status – I. Differenes in anti-oxidant profiles.** ***Comp Biochem Physiol B 1992;103:737***

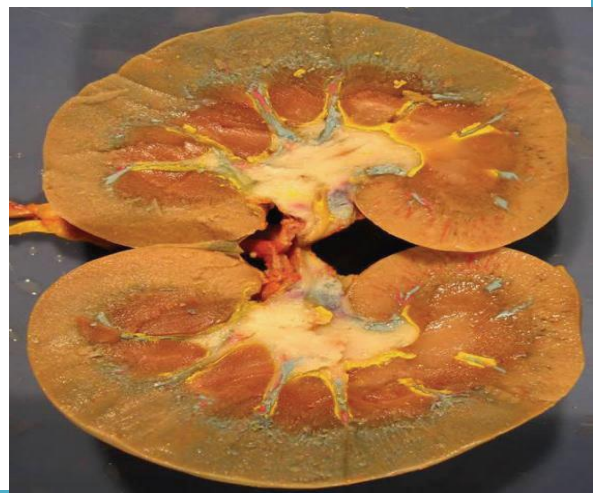


Anatomical and Physiological Similarities of the Kidney

Mice, rats and rabbits	Pig , monkeys and HUMANS
• Unilobular, unipapillar kidney • Urine empties directly into renal pelvis (no branched calicael network) • Segmental arteries are bypassed	• Multilobular, multipapilar kidney • Urine empties into calicael network into renal pelvis • Interlobular and segmental arteries







Simmons, J Urol 2008;180:19

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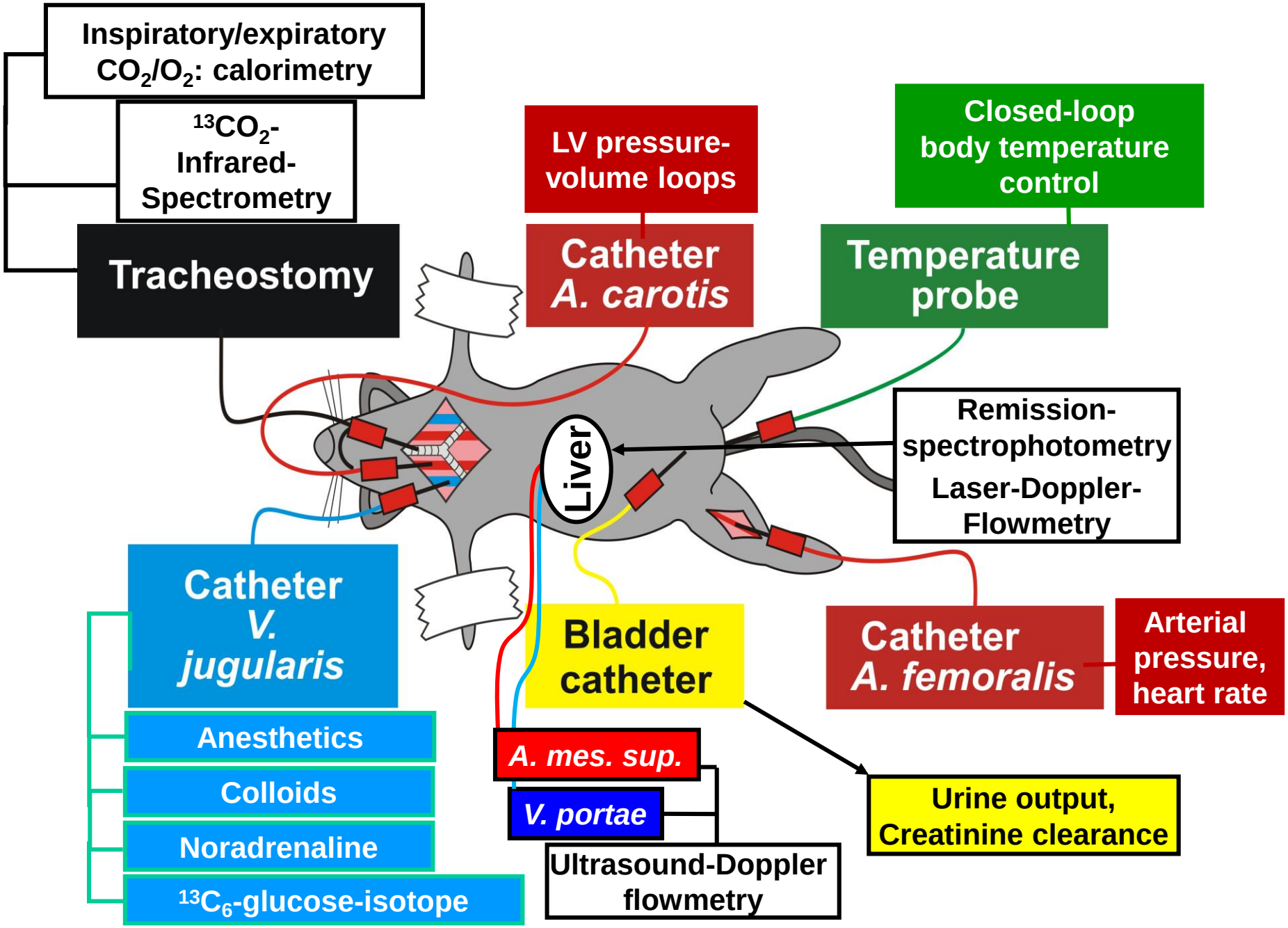
The „*Mouse Intensive Care Unit*“-concept


Inspiratory/expiratory CO_2/O_2: calorimetry
$^{13}CO_2$-Infrared-Spectrometry
Tracheostomy
LV pressure-volume loops
Catheter *A. carotis*
Closed-loop body temperature control
Temperature probe
Liver
Remission-spectrophotometry
Laser-Doppler-Flowmetry
Catheter *V. jugularis*
Anesthetics
Colloids
Noradrenaline
$^{13}C_6$-glucose-isotope
Bladder catheter
A. mes. sup.
V. portae
Ultrasound-Doppler flowmetry
Catheter *A. femoralis*
Arterial pressure, heart rate
Urine output, Creatinine clearance

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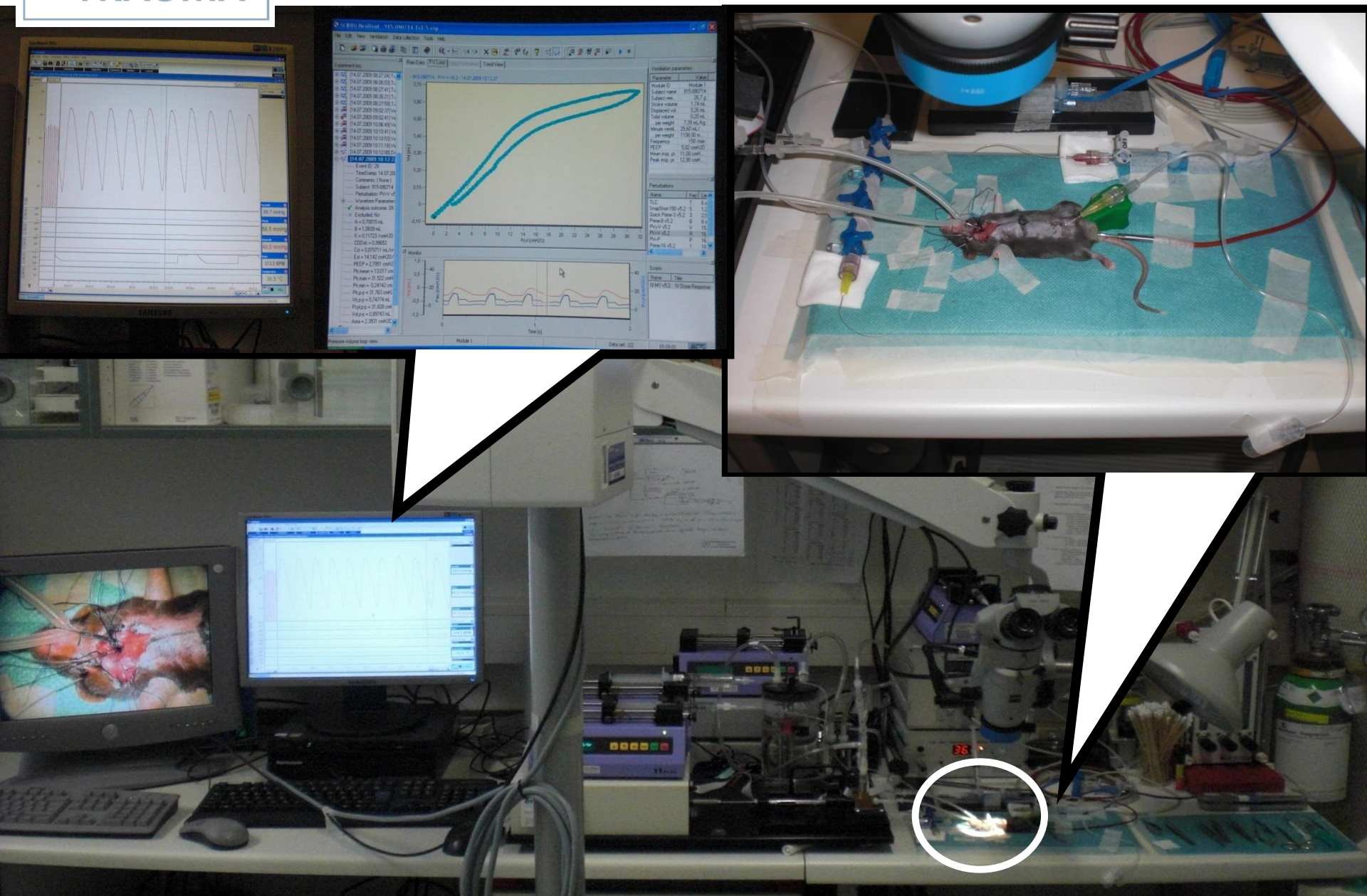
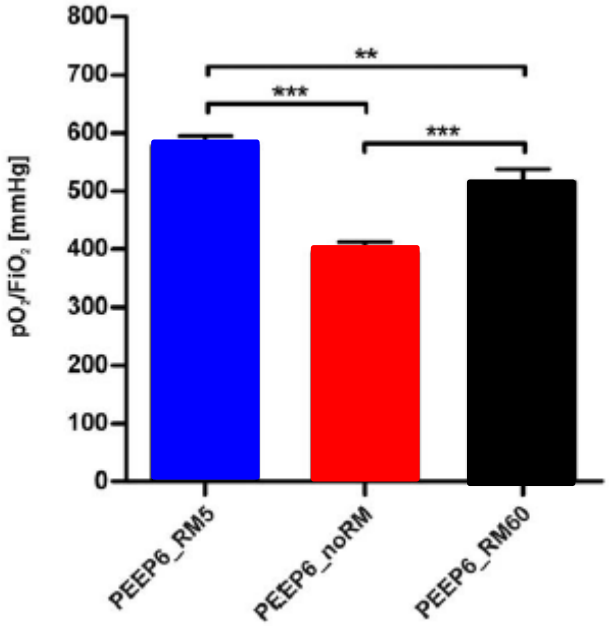


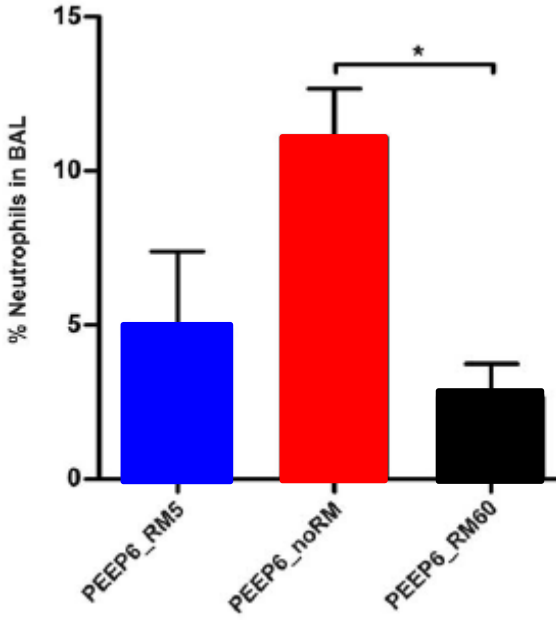
Recurrent Recruitment Manoeuvres Improve Lung Mechanics and Minimize Lung Injury during Mechanical Ventilation of Healthy Mice

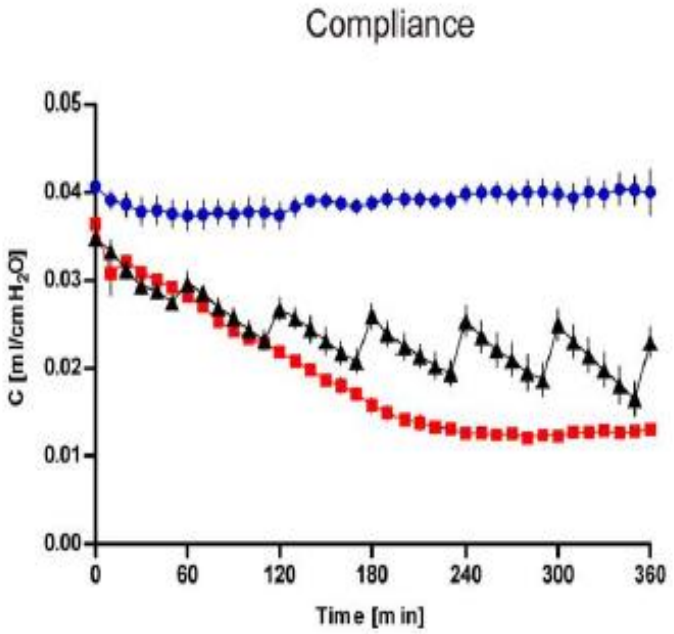
PLoS ONE 6(9): e24527, 2011

Lucy Kathleen Reiss*, Anke Kowallik, Stefan Uhlig

Mice, 6 hrs; FiO_2 0.5, RR 180/min, Vt 8 mL/kg, PEEP 6 cmH_2O, Recruitment 30 cmH_2O/1 sec every *5* / *60* min vs. *no recruitment*





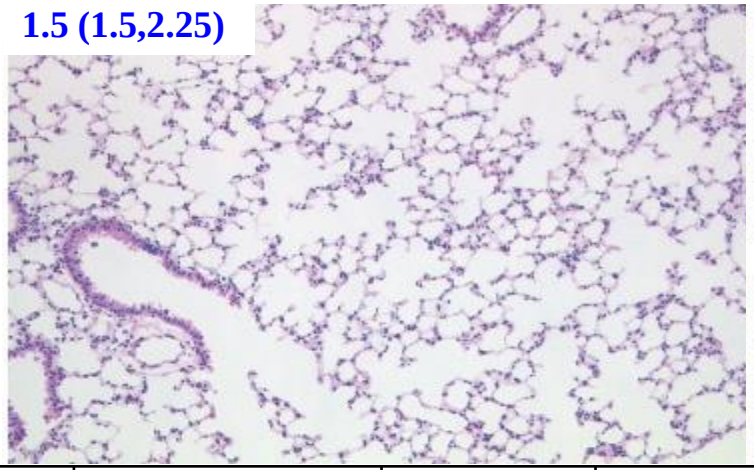


Wagner et al: Cardiopulmonary, Histologic, and Inflammatory Effects of Intravenous Na_2S After Blunt Chest Trauma-Induced Lung Contusion in Mice

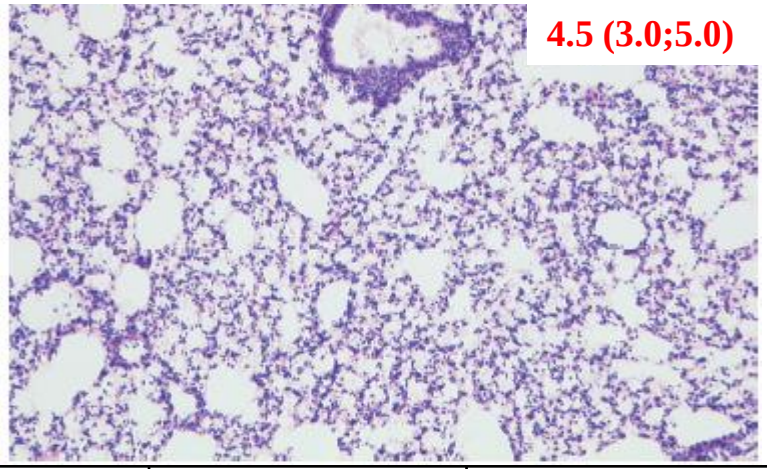
J Trauma. 2011;71: 1659

Total lung injury score (Σ dystelectasis/atelectasis + edema/hemorrhage + leukocyte infiltration + thickened alveolar membrane)

Sham **1.5 (1.5,2.25)**

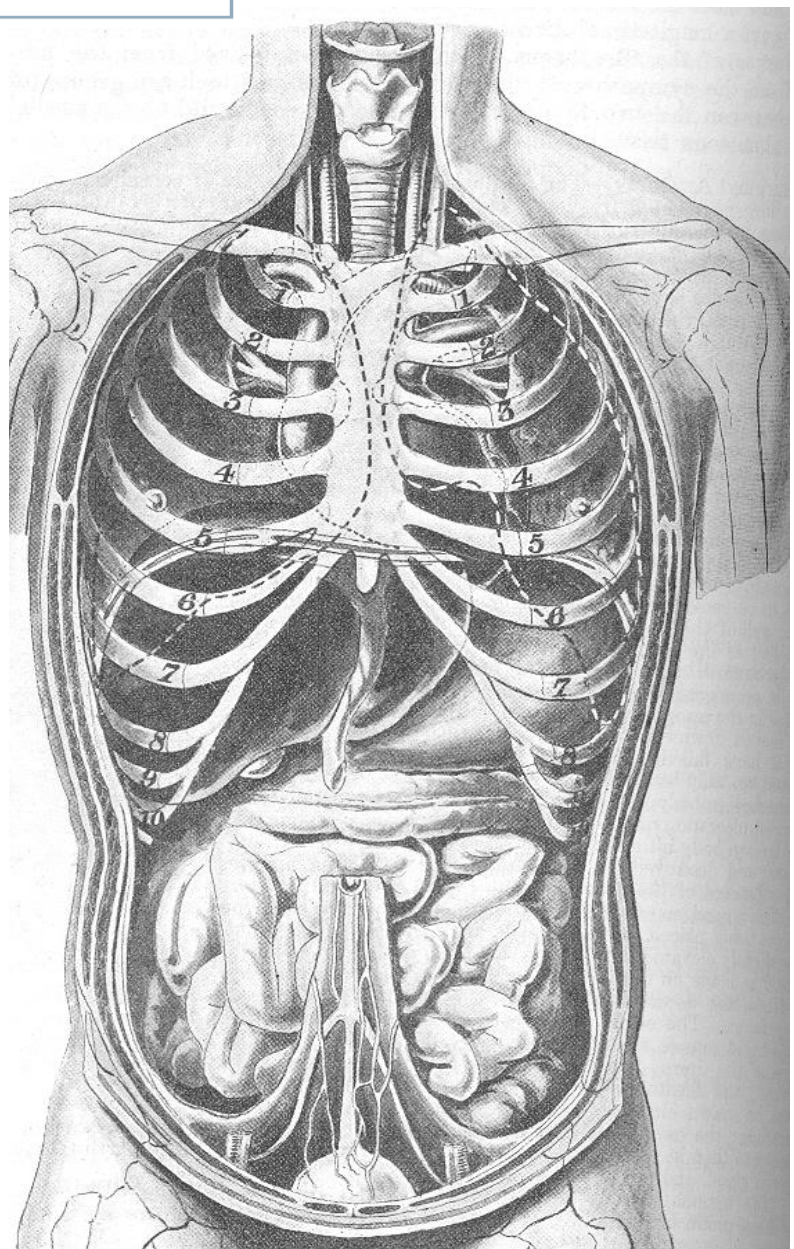


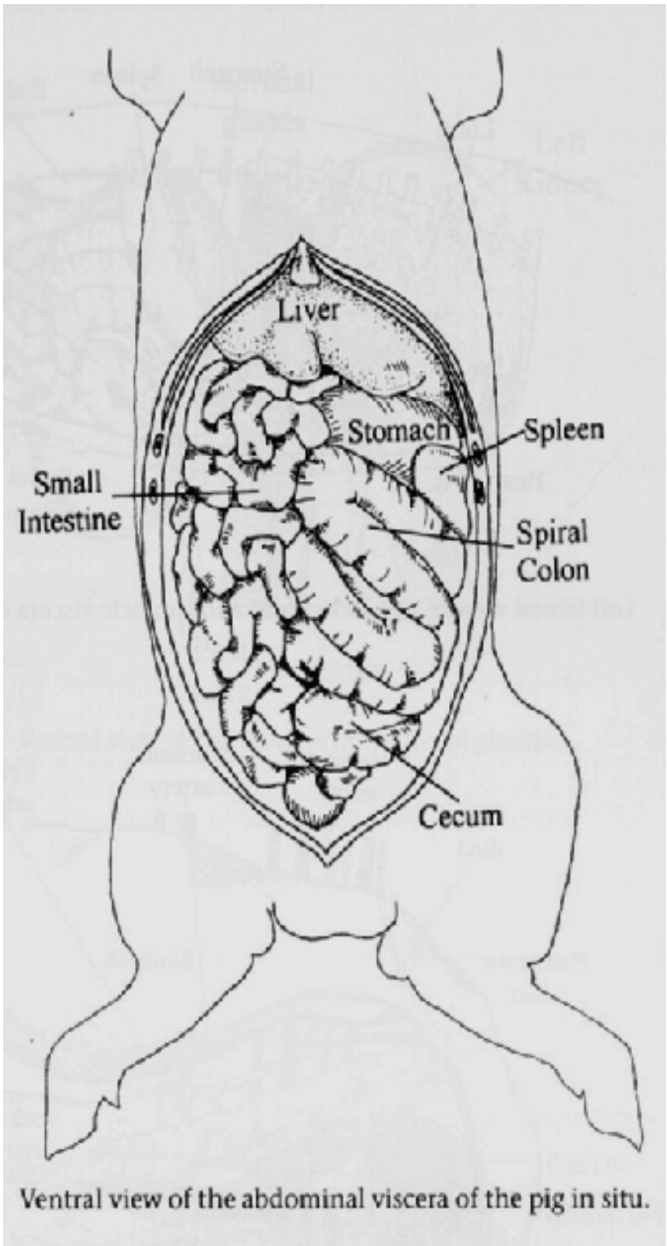
TxT **4.5 (3.0;5.0)**



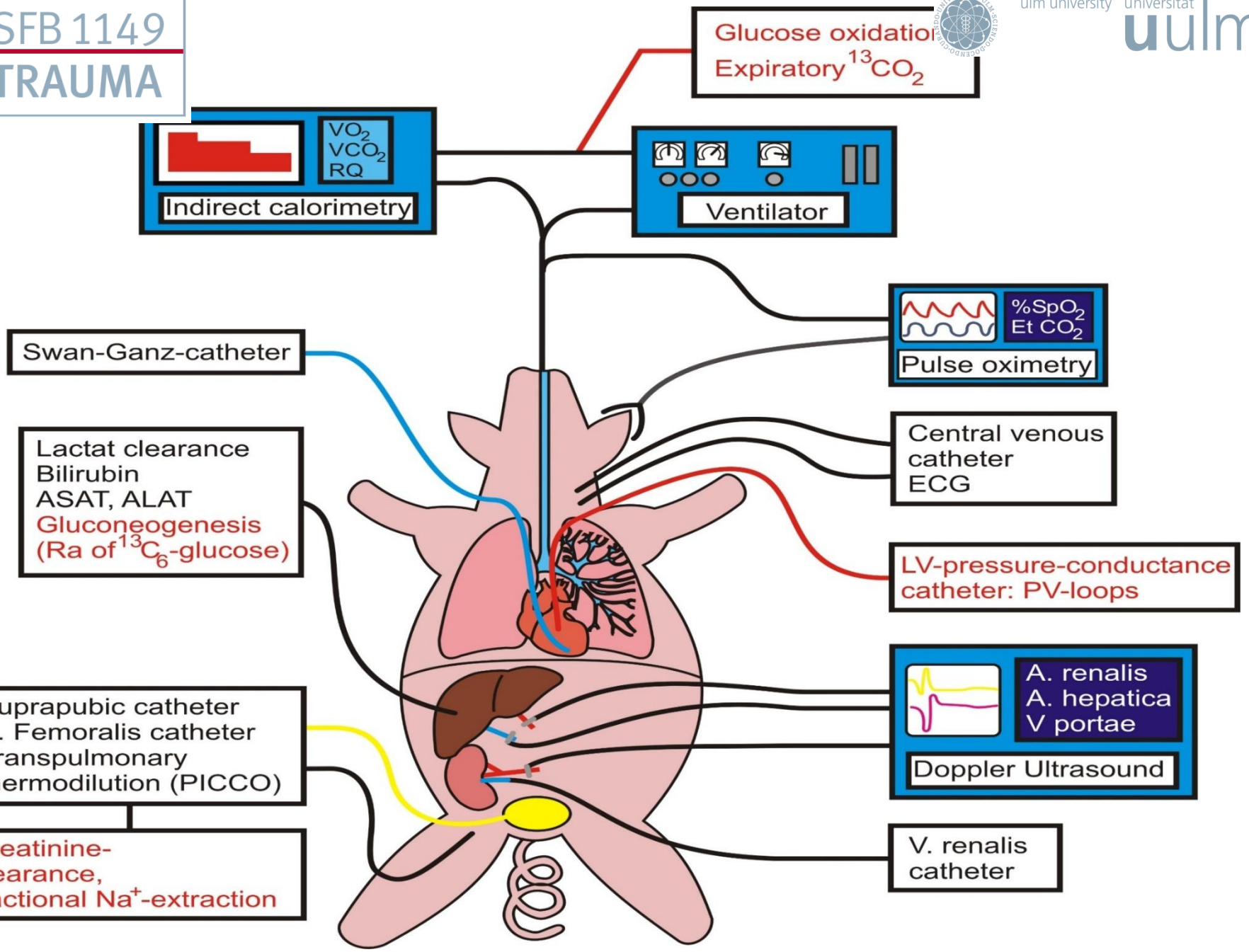
	Hert rate (beats/min)	MAP (mmHg)	pH	Cpl (µL/cmH_2O)	PaO_2/FiO_2 (mmHg)
Start	**325** (311;350)	**60** (58;71)	**7.31** (7.27;7.33)	**64** (60;71)	**362** (314;510)
End (4 h)	**370** (340;441)	**72** (66;78)	**7.38** (7.35;7.42)	**70** (64;81)	**564** (456;614)

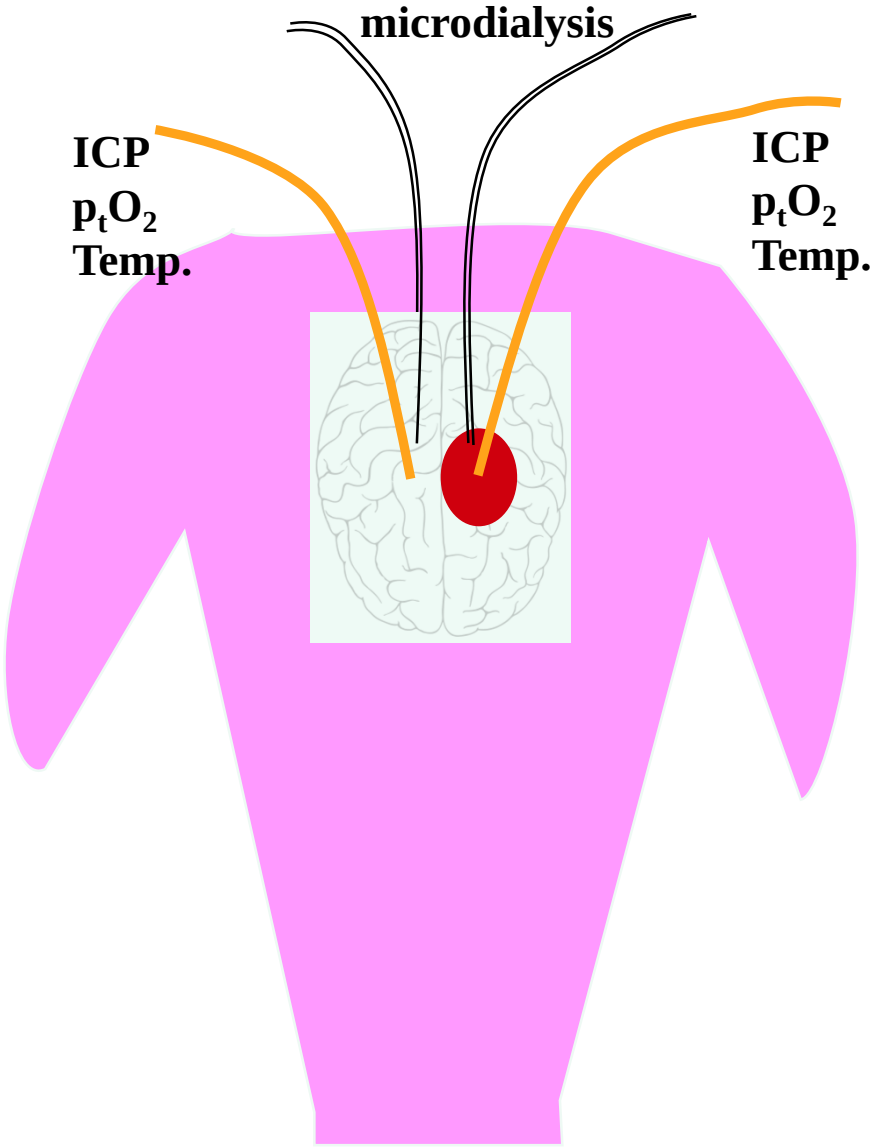
The „*Pig Intensive Care Unit*“-Concept





Ventral view of the abdominal viscera of the pig in situ.


Glucose oxidation
Expiratory $^{13}CO_2$
VO_2
VCO_2
RQ
Indirect calorimetry
Ventilator
%SpO_2
Et CO_2
Pulse oximetry
Swan-Ganz-catheter
Central venous catheter
ECG
Lactat clearance
Bilirubin
ASAT, ALAT
Gluconeogenesis
(Ra of $^{13}C_6$-glucose)
LV-pressure-conductance catheter: PV-loops
A. renalis
A. hepatica
V portae
Doppler Ultrasound
Suprapubic catheter
A. Femoralis catheter
Transpulmonary thermodilution (PICCO)
V. renalis catheter
Creatinine-clearance,
fractional Na^+-extraction

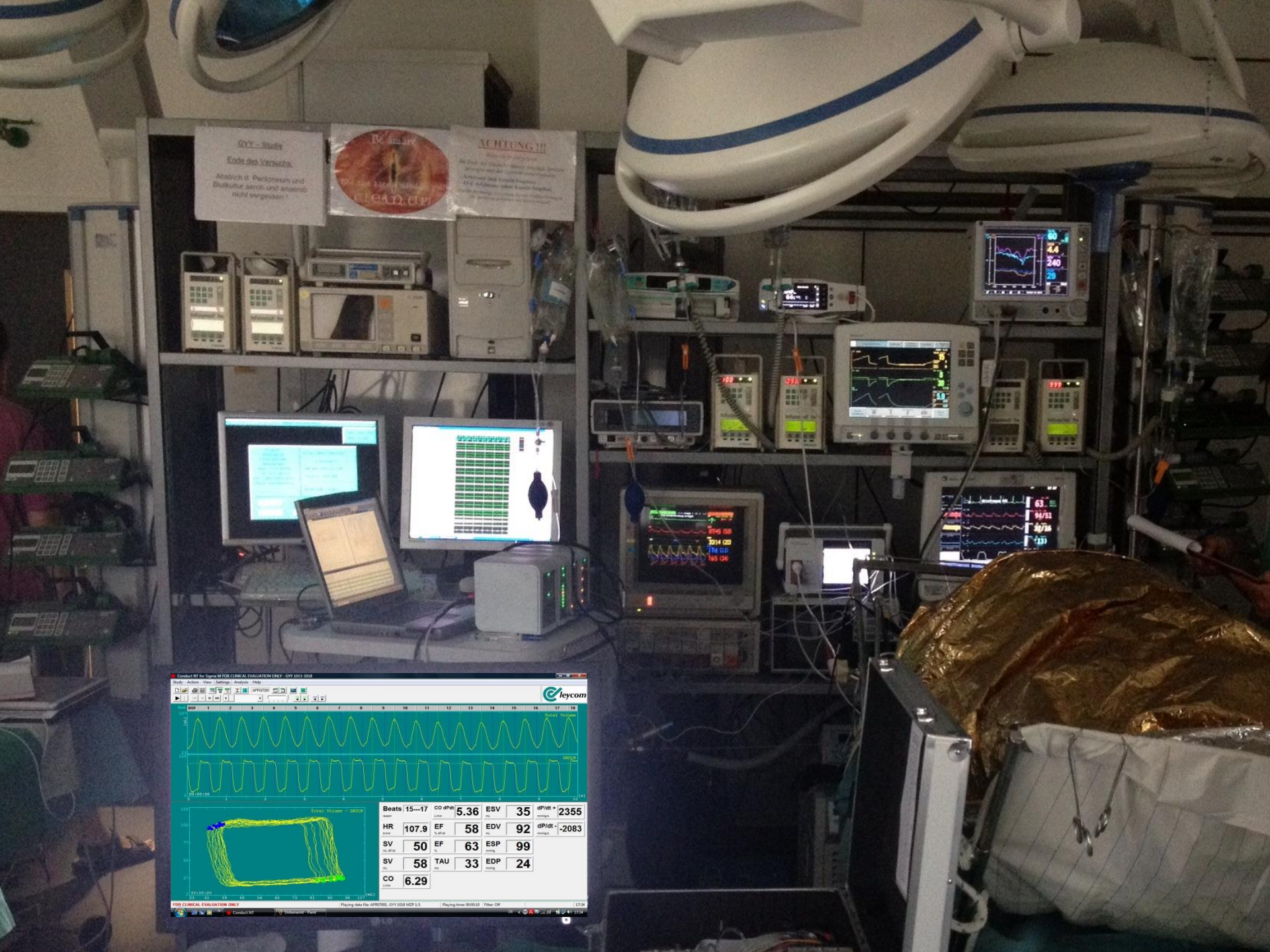


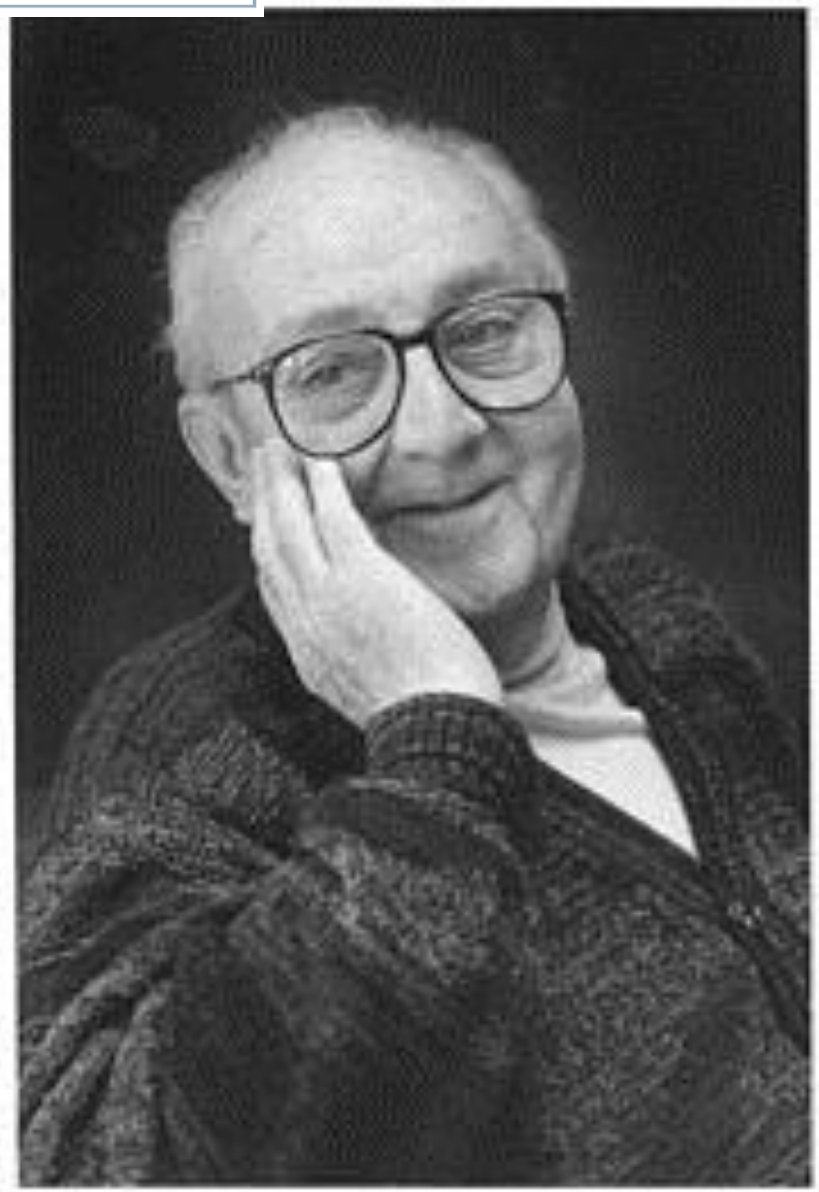
Measurements:

- **Modified Glasgow Coma Scale**
- **Response to painful stimuli**
- **Microdialysis for glutamate, pyruvate, lactate and glucose**
- **bilateral measurement of ICP (CPP), p_tO_2 and temperature**
- **Mitochondrial respiration and ROS production in PBMCs and neutrophils**
- **Electron spin resonance**

Post-mortem:

- **Nissl-Staining**
- **Western blots**
- **Immunohistochemistry**
- **High resolution respirometry**


GYY - Studie
Ende des Versuchs:
Abstrich d. Peritoneum und Blutkultur aerob und anaerob nicht vergessen !
ACHTUNG !!!
Conduct NT for Sigma M FOR CLINICAL EVALUATION ONLY - GYY 1013-1018
leycom
Total Volume
DRUCK
Total Volume - DRUCK
Beats 15---17
HR 107.9
SV 50
SV 58
CO 6.29
CO dPdt 5.36
EF 58
EF 63
TAU 33
ESV 35
EDV 92
ESP 99
EDP 24
dP/dt + 2355
dP/dt - -2083
FOR CLINICAL EVALUATION ONLY



George E.P. Box, FRS (1919-2013):

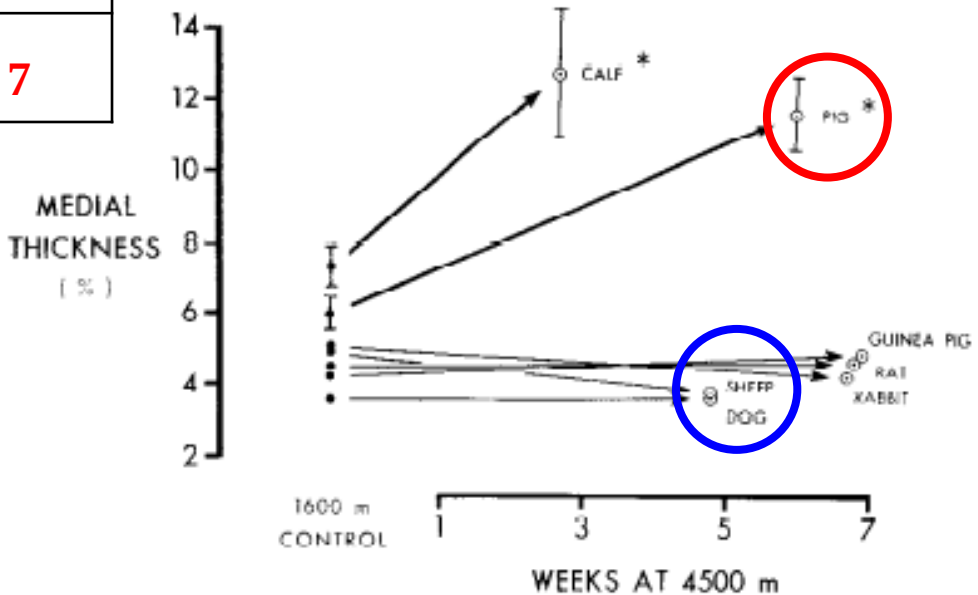
'All models are wrong, but some are useful'

Tucker A, et al:

Lung vascular smooth muscle as a determinant of pulmonary hypertension at high altitude.

Am J Physiol 1975;228:762

Species	Control	Altitude (4500m)
Sheep 42 days	**20** ± 1	**23** ± 2
Dog 42 days	**26** ± 2	**28** ± 1
Pig 42 days	**27** ± 1	**72** ± 7



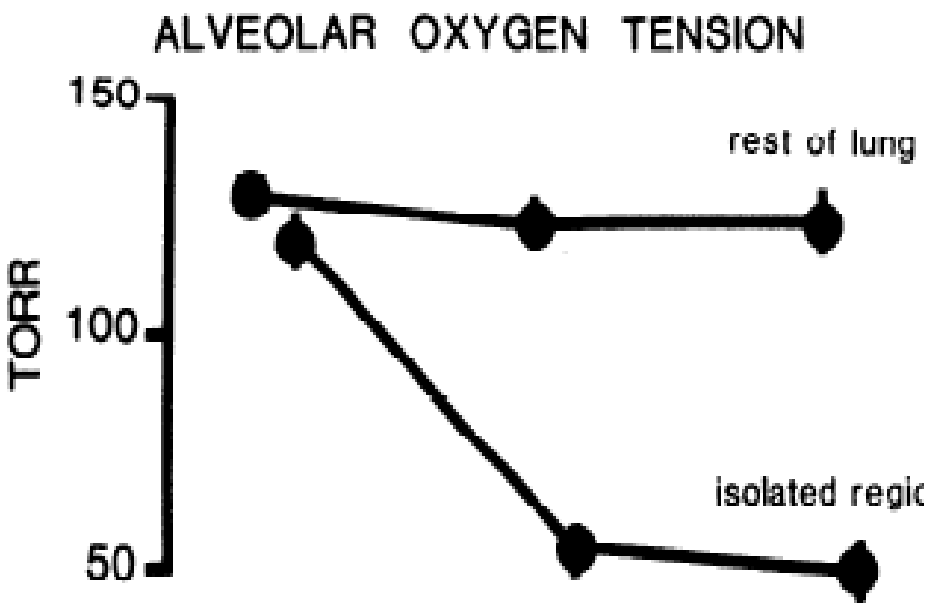
Kuriyama , et al:

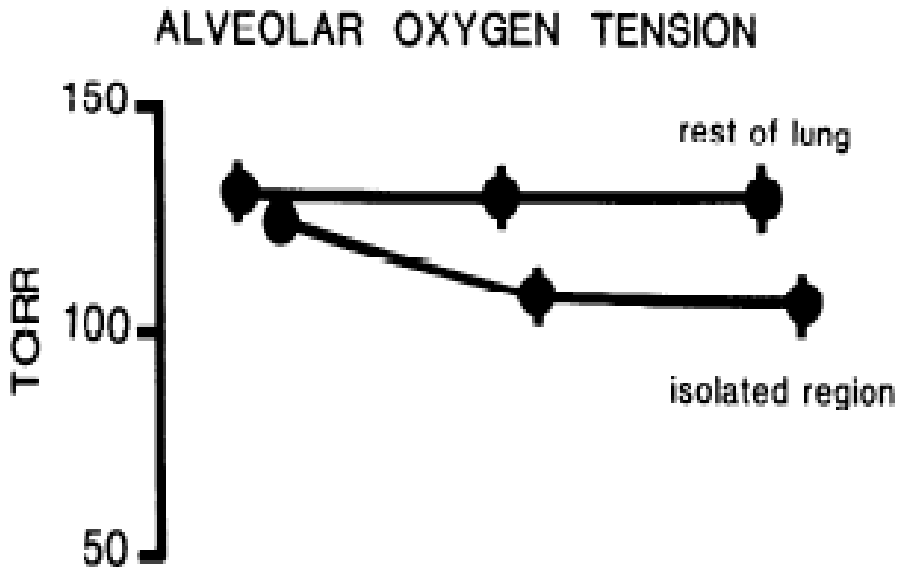
Role of collateral ventilation in ventilation-perfusion imbalance.

J Appl Physiol 1984;56:1500

Dogs, pigs; isolated lung region FiO_2 13 %, remainder 30 %:

Presence or not of alveolar collateral ventilation





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Do we need the „Animal ICU“?

Large vs. small animal models?

Both!

Pre-existing co-morbidity?

Translation to the clinics?

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How long should animal models last?

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Su et al:

Epidemiology of sepsis in Germany: results from a national prospective multicenter study.

Intensive Care Med 2007;33:606-18

ICU length of stay 12 - 19 days

Jesús Villar
Jesús Blanco
José Manuel Añón
Antonio Santos-Bouza
Lluís Blanch
Alfonso Ambrós
Francisco Gandía
Demetrio Carriedo
Fernando Mosteiro
Santiago Basaldúa
Rosa Lidia Fernández
Robert M. Kacmarek
on behalf of the ALIEN Network

The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation

Intensive Care Med (2011) 37:1932

Hospital	ICU	Mechanical Ventilation
35 (19-64)	22 (13-36)	17 (10-29)

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Recurrent Recruitment Manoeuvres Improve Lung Mechanics and Minimize Lung Injury during Mechanical Ventilation of Healthy Mice

PLoS ONE 6(9): e24527

Lucy Kathleen Reiss*, Anke Kowallik, Stefan Uhlig

Experimental design				Monitored parameters			
Ventilation	V_T [ml/kg]	PEEP [cmH_2O]	RM	Lung functions	BGA	BP/cardiac activity	SpO_2
30 mi	7/10	2/0	*	+	–	–/P	+
60 mi	8	6/3	**	+	–	–	–
140 m	30/10	2/0	2x/min	+	+	–/ECG	–
150 m	8	6/2	1x/5 min or 1x/75 min	+	–	–/P	+
4 h	25/7	–	–	+	–	–/ECG	–
4 h	20/6	2	–				
4 h	30	–	–				
4 h	20/7						
4 h	8						
5 h	30/6	–	–				
5 h	15/7.5	2	–	–	+	+/+	–
6 h	24	–	–	–	–	+/P	–
6 h	12	6	*	–	+	+/P	+
4 h/8	20/10	2	1x/h	–	–	+/–	–
8 h	12	2	–	–	+	+/–	–
6 h	16/8	2	1x/5 min or 1x/60 min or no RM	+	+	+/ECG, P	+

30 min – 8 h (- 16 h)

Szabari et al:

Relation between respiratory mechanics, inflammation, and survival in experimental mechanical ventilation.

AJRCMB 2018 doi: 10.1165/rcmb.2018-0100OC

Benes et al: **Searching for mechanisms that matter in early septic acute kidney injury: an experimental study.** ***Crit Care 2011;15:R256*** **swine** ***22h***

Su et al: **Effects of a selective iNOS inhibitor versus norepinephrine in the treatment of septic shock.** ***Shock 2010;34:243-9*** **sheep** ***24h***

Lange et al: **Time course of nitric oxide synthase, nitrosative stress, and poly(ADP ribosylation) in an ovine sepsis model.** ***Crit Care 2010;14:R129*** ***24h***

Vuda et al: **Effects of catecholamines on hepatic and skeletal muscle mitochondrial respiration after prolonged exposure to faecal peritonitis in pigs.** ***Innate Immun 2011;1-14*** ***27h***

Correa et al: **Increasing mean arterial blood pressure in sepsis: effects on fluid balance, vasopressor load and renal function.** ***Crit Care 2013;17:R21*** **swine** ***48h***

Horst et al: **Characterization of blunt chest trauma in a long-term porcine model of severe multiple trauma.** ***Sci Rep 2016;6:39659*** ***72h***

Esechie et al: **Beneficial effect of hydrogen sulphide donor (sodium sulphide) in an ovine model of burn- and smoke-induced acute lung injury.** ***Br J Pharmacol 2009;158:1442-53*** ***96h***

Bogdanski et al: **Cerebral histopathology following portal venous infusion of bacteria in a chronic porcine model.** ***Anesthesiology 2000;93:793-804*** ***104h***

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Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

As long as possible!?!

Translation to the clinics?

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Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?

Severe Sepsis and Septic Shock

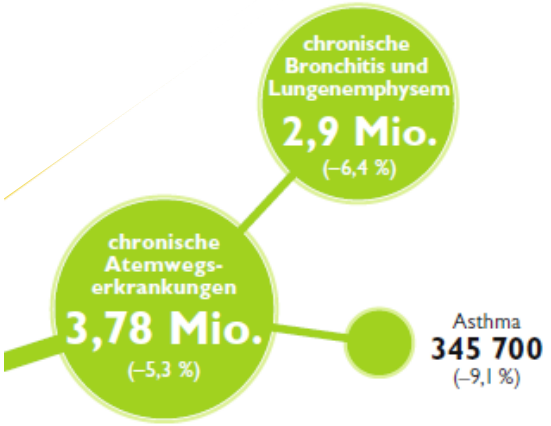
Derek C. Angus, M.D., M.P.H., and Tom van der Poll, M.D., Ph.D.

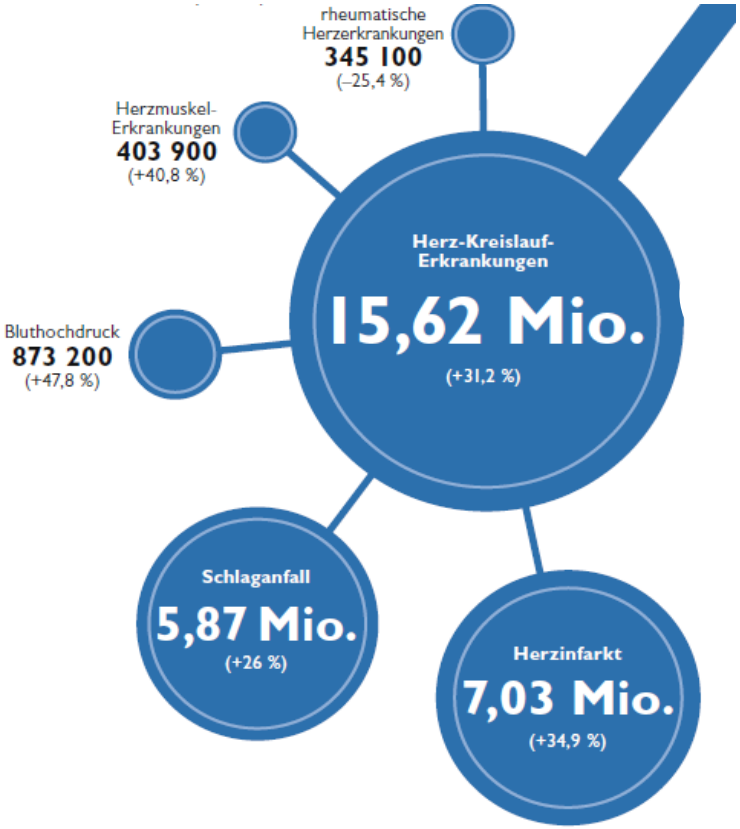
N Engl J Med 2013;369:840-51

„.... For example, options to enrich the pre-clinical portfolio include the study of animals that are more genetically diverse, are older, or have preexisting disease. ...."

7. FEBRUAR 2013 DIE ZEIT N°7

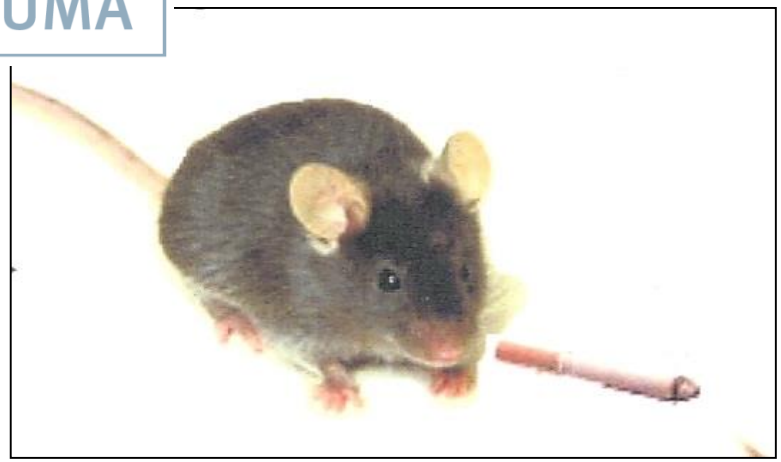
Woran wir sterben



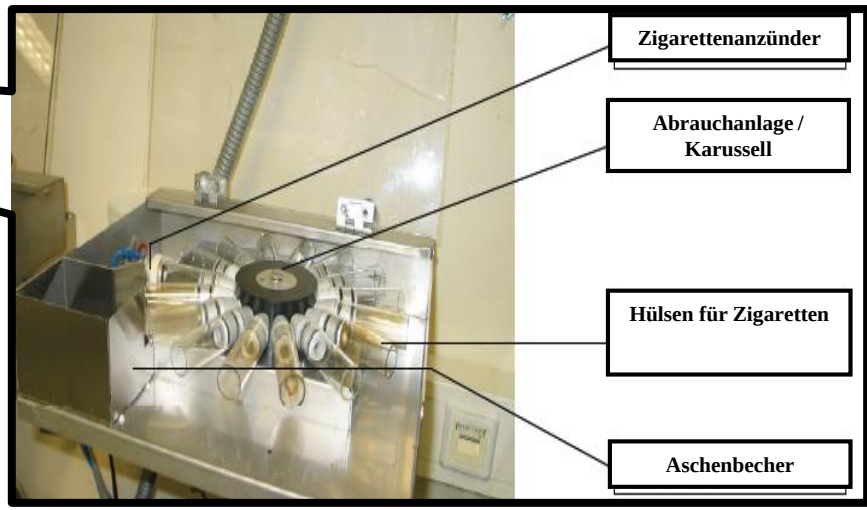


Unter dem Titel »Global Burden of Disease« wurden erstmals 1992 alle Menschen erfasst, die innerhalb des Jahres gestorben waren. Außerdem beschrieb die Studie die wichtigsten Risikofaktoren für die Gesundheit. Vor einem Monat wurde nun, zwanzig Jahre später, eine Folgestudie veröffentlicht, basierend auf den knapp 53 Millionen Todesfällen des Jahres 2010. Das Fazit: Die Bevölkerung ist in der Zwischenzeit deutlich gesünder geworden. Infektionskrankheiten wurden zurückgedrängt, dafür sterben mehr Menschen an Altersgebrechen und Zivilisationsleiden

Stroke $5.8 \cdot 10^6$	**24 %**
MI $7.0 \cdot 10^6$	
COPD $3.8 \cdot 10^6$	**7 %**
Diabetes $1.3 \cdot 10^6$	**2.5 %**



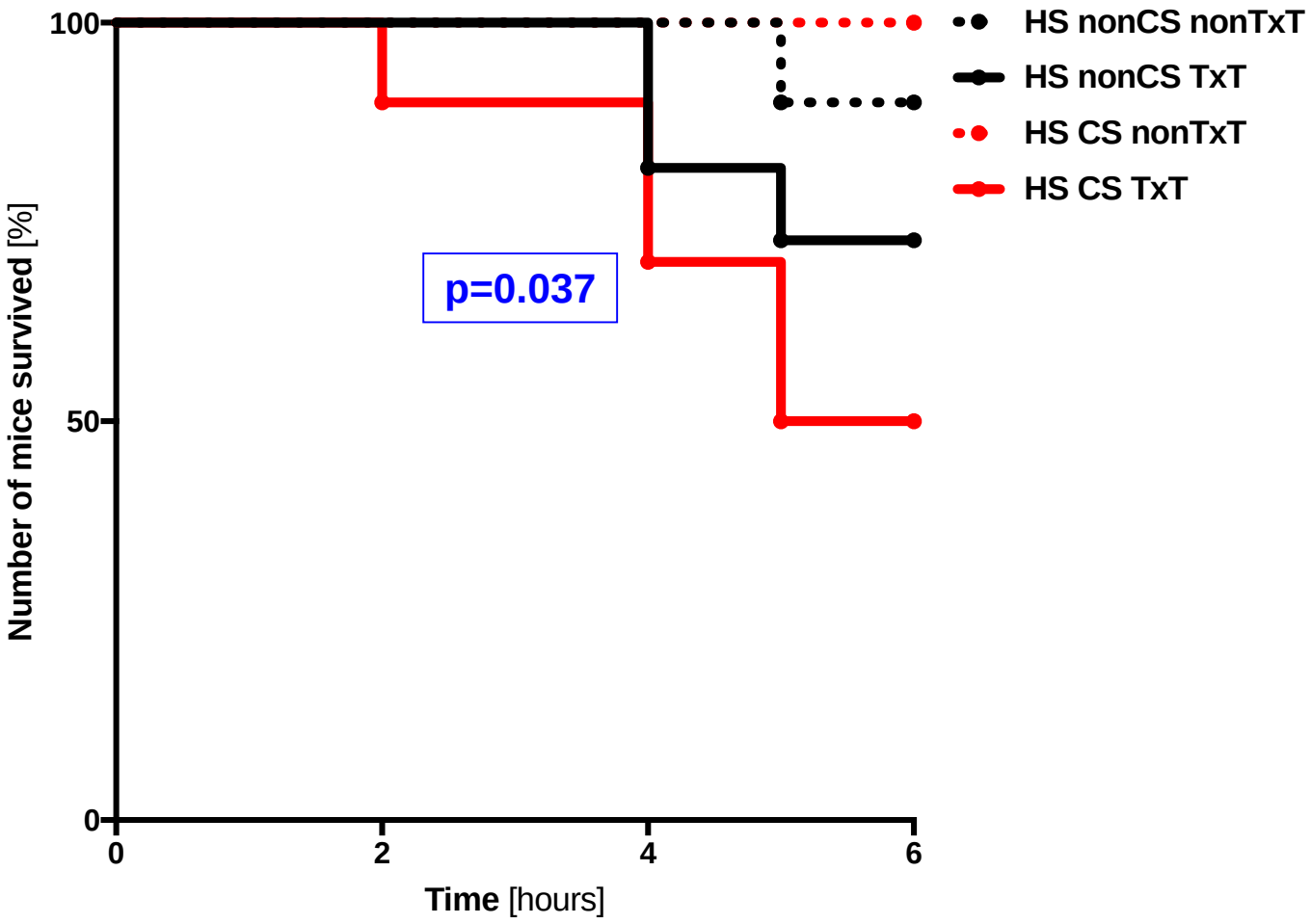
Roth Händle
4 days / week over 3-4 weeks



Hartmann et al:

In-depth characterization of the effects of cigarette smoke exposure on the acute trauma response and haemorrhage in mice.

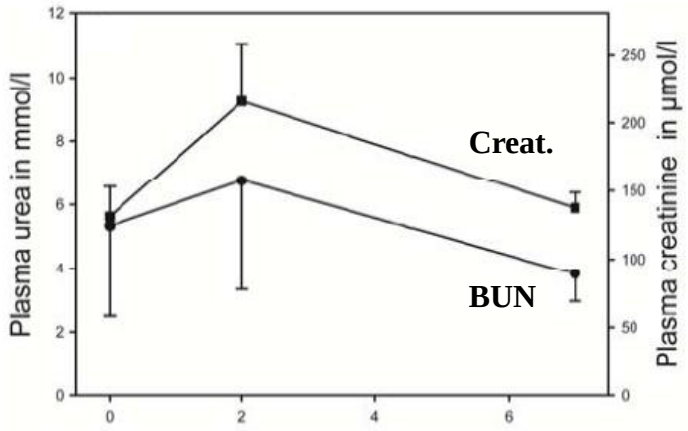
Shock, doi: 10.1097/SHK.0000000000001115

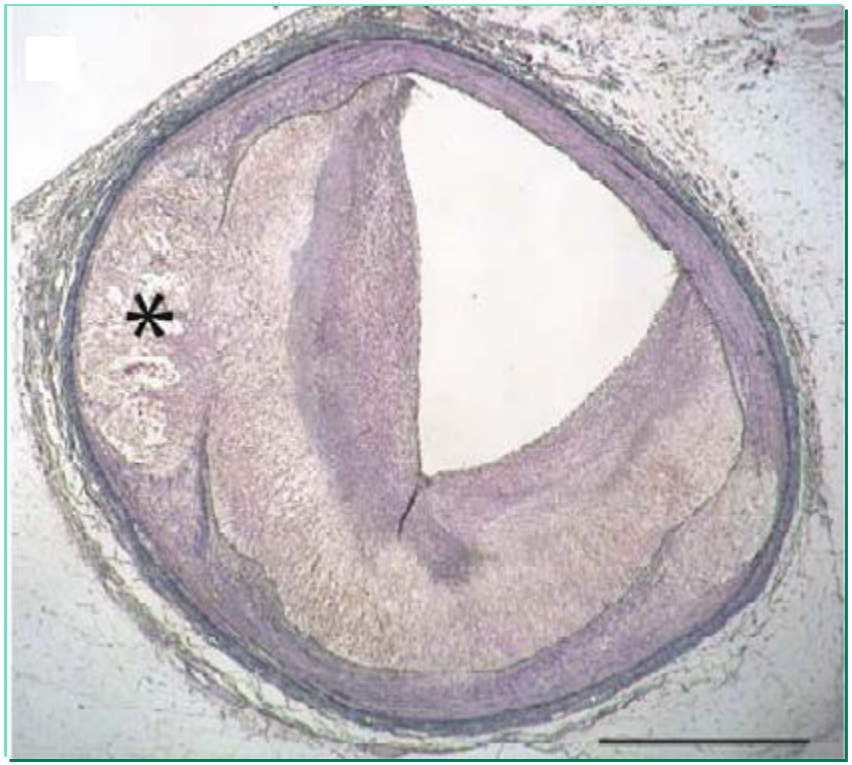


DANISH MEDICAL BULLETIN

Human-like atherosclerosis in minipigs: a new model for detection and treatment of vulnerable plaques

Troels Thim

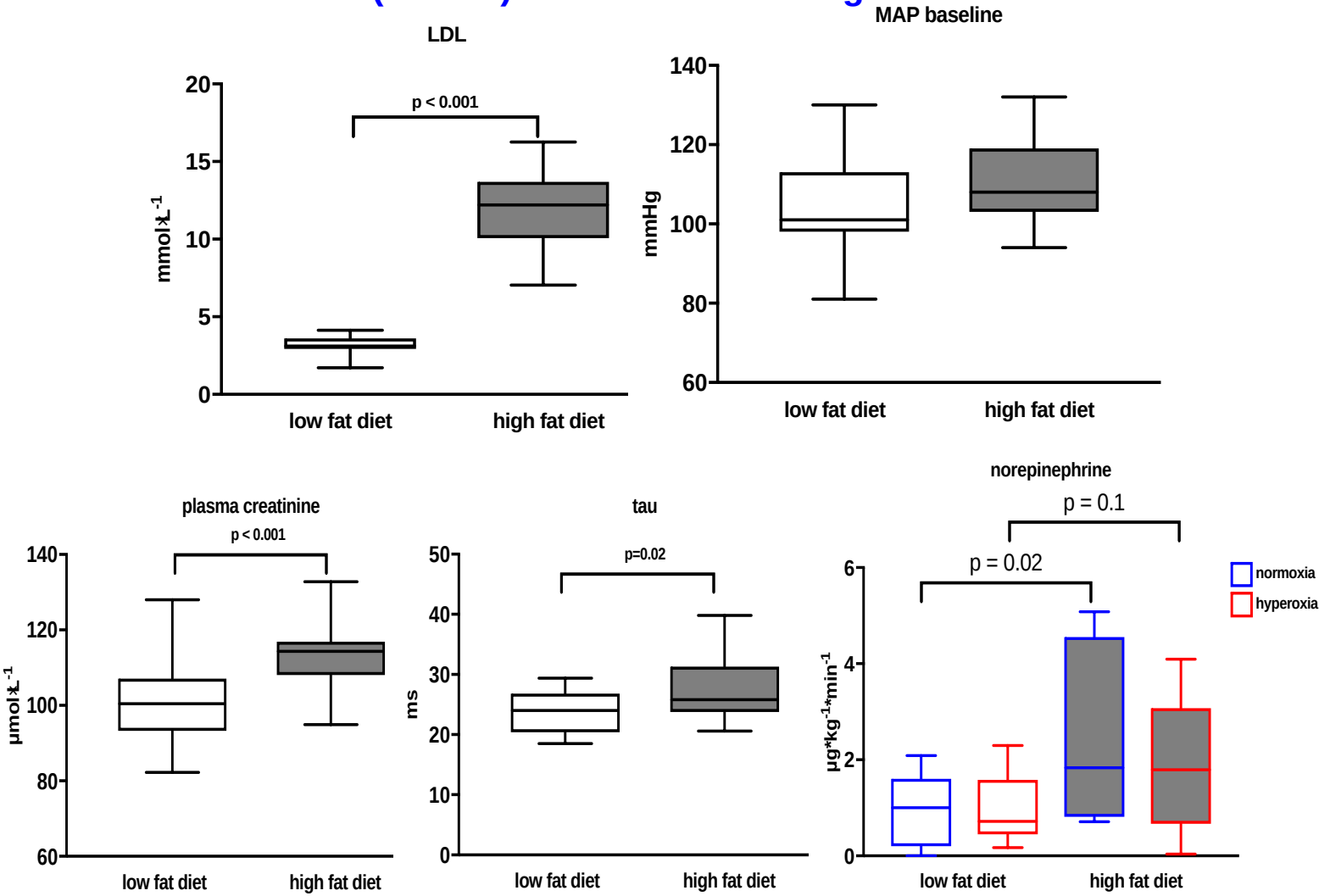




Hartmann et al: Effects of hyperoxia during resuscitation from hemorrhagic shock in swine with pre-existing coronary artery disease.

Crit Care Med 2017;45:e1270-9

Pigs with pre-existing atherosclerosis: FiO2 1.0 vs. standard treatment during the first 24 h (of 48 h) after 3-h hemorrhage



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Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

Pre-existing co-morbidity?

Must be included!

ULM SFB 1149 TRAUMA

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Do we need the „Animal ICU“?

Large vs. small animal models?

How long should animal models last?

Pre-existing co-morbidity?

Translation to the clinics?

Träger et al.

Norepinephrine and *N*ω-monomethyl-L-arginine in porcine septic shock: effects on hepatic O_2 exchange and energy balance.

Am J Respir Crit Care Med 1999;159:1758

In summary, L-NMMA and NOR were equally effective in maintaining MAP during long-term hyperdynamic porcine endotoxic shock.

López et al.

Multi-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: Effect on survival in patients with septic shock.

CCM 2004;32:21

Conclusions: **In this study, the nonselective nitric oxide synthase inhibitor 546C88 increased mortality in patients with septic shock.**

Simon et al.

Comparison of cardiac, hepatic and renal effects of arginine vasopressin and noradrenaline during porcine fecal peritonitis: a randomized, controlled trial. *Crit Care 2009;13:R113*

Low-dose AVP appears to be safe with respect to myocardial function and heart injury and even attenuates kidney and liver dysfunction and tissue damage during well-resuscitated porcine septic shock.

Gordon et al.

Effect of early vasopressin vs. norepinephrine on kidney failure in patients with septic shock. The VANISH randomized clinical trial. *JAMA 2016;316:509*

....Although these findings do not support the use of vasopressin to replace norepinephrine as initial treatment in this situation, the confidence interval included a potential clinically important benefit for vasopressin....

Barth et al*. Effects of ventilation with 100 % oxygen during early hyperdynamic porcine fecal peritonitis. *CCM 2008;36:495

Hauser et al*. Hemodynamic, metabolic and organ function effects of pure O_2 ventilation during established fecal peritonitis-induced septic shock. *CCM 2009;37:2465

Conclusions: **During early hyperdynamic porcine septic shock, 100% oxygen improved organ function and attenuated tissue apoptosis without affecting lung function and oxidative or nitrosative stress. Therefore, it might be considered as an additional measure in the first phase of early goal-directed therapy.**

Conclusions: **When compared with the previous report on hyperoxia initiated simultaneously with induction of sepsis, i.e., using a pretreatment approach, pure oxygen ventilation started when porcine fecal peritonitis-induced septic shock was fully developed proved to be equally safe with respect to lung function and oxidative stress, but exerted only moderate beneficial effects.**

Asfar et al*. Hyperoxia and hypertonic saline in patients with septic shock: A randomized clinical trial. *Lancet Respir Med 2017;5:180

…. total SOFA was significantly lower by day 7 (table 2) and the liver SOFA score was significantly lower from day 3 to day 7 …. In conclusion, in patients with septic shock, hyperoxia and hypertonic saline used as fluid resuscitation did not reduce the mortality at either 28 days or 90 days. ….

***Osuchowski et al*.**

Minimum quality threshold in pre-clinical sepsis studies (MQTiPSS): an international expert consensus initiative for improvement of animal modeling in sepsis.

Intensive Care Med Exp 2018;6:26

Zingarelli et al.

Part I: Minimum Quality Threshold in Preclinical Sepsis Studies (MQTiPSS) for Study Design and Humane Modeling Endpoints.

Shock 2019;51:10-22

Libert et al.

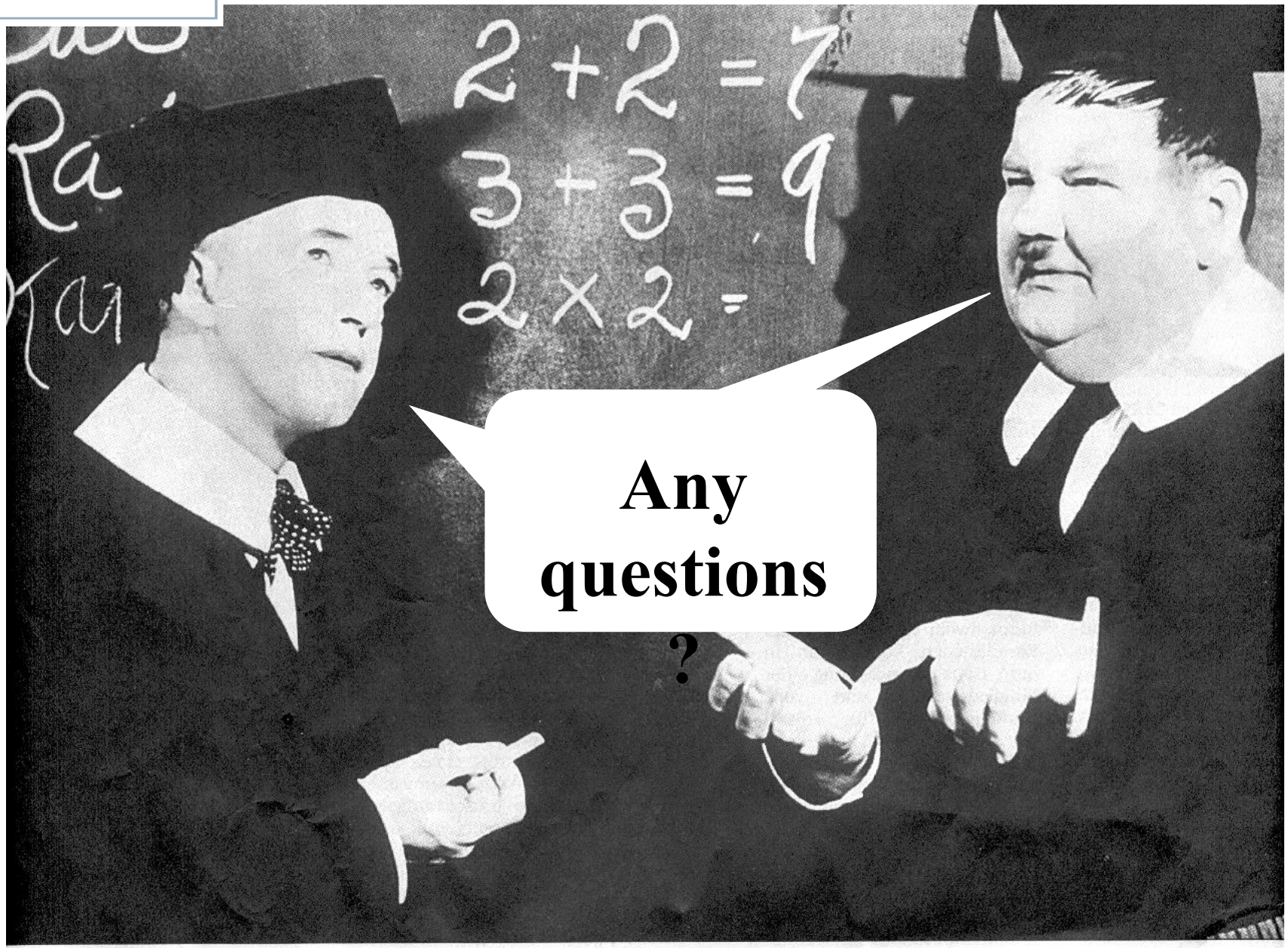
Part II: Minimum Quality Threshold in Preclinical Sepsis Studies (MQTiPSS) for Types of Infections and Organ Dysfunction Endpoints.

Shock 2019;51:23-32

Hellman et al.

Part III: Minimum Quality Threshold in Preclinical Sepsis Studies (MQTiPSS) for Fluid Resuscitation and Antimicrobial Therapy Endpoints.

Shock 2019;51:33-43


2+2=7
3+3=9
2×2=
Any questions ?