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PLASMATIC CYCLIC GUANOSINE 3',5' MONOPHOSPHATE (cGMP) IS NOT A DIRECT INDEX OF THE IN VIVO NITRIC OXIDE ENHANCED PRODUCTION IN HUMAN SEPTIC SHOCK (SS).

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Cyclic GMP is a stable nucleotide resulting from guanylyl cyclases (GC) activation. In the cardiovascular system there are 2 isoenzymes of GC : a particulate form located on endothelial cells and activated by atrial natriuretic peptides (ANP), and a soluble form present in platelets, endothelial cells and vascular smooth muscle cells as well, which is activated by nitric oxide (NO). This free radical has now been involved in the loss of vascular responsiveness to catecholamines in human SS.

Aim of the study : to evaluate whether plasmatic cGMP concentrations are increased or not during human hyperkinetic SS and to investigate cGMP correlationship with hemodynamic and biological data.

Patients and methods : hemodynamic data, plasmatic cGMP and ANP, creatininemia and platelets counts were simultaneously recorded twice a day during the first 72 hours of hospitalization in 22 patients requiring full ressucitation for hyperdynamic SS (CI = 5.18 ± 0.32 $l.min^{-1}.m^{-2}$, SVR = 1 016 ± 112 $dyne.s.cm^{-5}$). Data are given as mean ± SEM and are compared to those from healthy volonteers by a non parametric analysis of variance (Kruskall-Wallis).

Results :

1. Patients with SS had significantly higher cGMP levels when compared to healthy controls at admission time (11.84 ± 1.52 versus 1.77 ± 0.18 $pmol.ml^{-1}$, $p<0.0001$) and overall the survey.
2. The same result was observed with ANP (64.9 ± 7.8 versus 13 ± 1.02 $pg.ml^{-1}$, $p<0.0003$).
3. cGMP negatively correlated with SVR ($p<0.005$) but positively with thrombopenia ($p<0.0001$), creatininemia ($p=0.0001$) and with ANP as well ($p<0.01$).

Conclusions : These results demonstrate that a significant increase in GC activity occurs *in vivo* in human SS and show that it involves not only particulate but also soluble GC. These data do not support the notion that, in these conditions, plasmatic cGMP might become a direct index of nitric oxide production.

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AMPLIFICATION OF CYTOKINE RELEASE FOLLOWING CONTACT BETWEEN LPS-MACROPHAGES AND VASCULAR TISSUE.

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Cytokines are involved in septic vasoplegia and play a key role in inflammatory processes as a result of interactions between marophages (MF) and vascular cells. We have already shown that LPS activated macrophages depress markedly rabbit carotid contractility in a model of in vitro perfused whole vessel (J. Clin. Invest. March 1992). Precise regulation is essential to preserve the host from cytokine-mediated injury. To investigate the secretory cooperation between MF and vascular cells, elicited peritoneal rabbit MF were incubated for 18h at 5.10^6/ml with LPS(1µg/ml) and then exposed for 4h to a challenge dose of LPS (1µg/ml) in flasks, or in the lumen of perfused and pressurised excised rabbit carotids. Supernatants from 18h LPS-activated MF and 4h reincubated MF, as well as cell-free intraluminal (i.l) solutions were biologically assayed for IL-1 and TNF, using D10.G4 T cell clone proliferation and L929 murine fibroblasts cytotoxicity, respectively. Results (mean+SEM) were:

	18h MF	4h reincub. MF	4h i.l. solution
IL-1 (U/ml)	360 ± 75 (n=16)	120 ± 21(n=7)	4400 ± 1400 (n=4)
TNF (U/ml)	2950± 750 (n=4)	27 ± 12 (n=3)	340 ± 82 (n=6)

Sequential exposure of MF to LPS in flasks led to an effective down regulation of TNF release. Yet, IL-1 production seemed to be less affected by the early phase of LPS tolerance, in support of differential regulation of IL-1 and TNF secretions. Strikingly, a marked increase in TNF, and even more, in IL-1 concentrations was found in i.l. solutions. This suggests that contact between MF and vascular tissue led to an amplification of the cytokine release, resulting from modification of MF phenotype and/or from stimulation of the vessel wall self-production. These results highlight the critical role of cell-to-cell interactions in the production of potentially deleterious monokines involved in septic shock.

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HIGH LEVELS OF PORTAL TNFα DURING ABDOMINAL AORTIC SURGERY IN MAN.

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During shock, translocation of bacteria and lipopolysaccharide (LPS) from the ischemic gut might occur and could explain the excess of cytokine production detectable in plasma during human multiple organ dysfunction syndrome. To verify this hypothesis, we studied the model of mild gut ischemia due to the aortic clamping in 14 patients undergoing abdominal aortic surgery (Group I). Levels of LPS, TNF and IL-6 were measured at Day-1, before clamping (T1), 15 mn (T2) and 3h (T3) after reperfusion, and at Day+1, 2, 3, and 5. Blood cultures, LPS and cytokine levels were compared in systemic and portal blood at T1 and T2. Patients undergoing carotid revascularization (Group II: n=6) were studied as control. Systemic levels of LPS, TNF and IL-6 of Group I patients are described in the table

*p<.05 vs D-1	D-1	T1	T2	T3	D1	D2	D3	D5
LPS(pg/ml)	O	7.7	21.3*	7.3	5.7	5	3.4	O
TNF(pg/ml)	O	217	83	299*	222*	238	279*	505*
IL-6 (U/ml)	26	11	66	95	106*	105*	61	14

Neither LPS or TNF were found in Group I and II patients before surgery nor after in Group II patients. All blood cultures were negative. In Group I, systemic endotoxin was found in 2 patients at T1, 9 at T2, 6 at T3, 3 at D3 and none at D5. Portal endotoxin was detectable in 36% of the patients before clamping and in 71% after reperfusion. Higher althought non significant LPS mean levels were observed in portal than in systemic blood at T2 (31±9 vs 21±7 pg/ml). Significantly higher levels of TNF were detected in portal than in systemic blood at T2 (293±84 vs 83±34 pg/ml, p=.002). Kinetics of IL-6 productions were similar in Group I and II, with a peak the day following surgery. In conclusion, 1) circulating IL-6 is mainly a marker of the surgical procedure. 2) In man, mild gut ischemia consecutive to bowel manipulation, aortic clamping and reperfusion leads to LPS but not bacterial translocation. 3) A lymphatique carriage of LPS could explain the lack of significance between portal and systemic levels of LPS. 4) The high levels of portal TNF indicate that a gut production occurs, suggesting that the gut-associated macrophages present in the lamina propria, activated by translocated LPS, could be a major source of circulating TNF.

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PREVENTION OF BACTERIAL TRANSLOCATION AND ENDOTOXAEMIA IN RATS WITH ZYMOSAN INDUCED PERITONITIS BY SELECTIVE DECONTAMINATION.

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The mucosa of the GI-tract provides an effective barrier to the entry of intestinal bacteria and endotoxins. This barrier function can be lost in critical illness and thus may lead to translocation of bacteria and endotoxins, resulting in bacteraemia, endogenous infections and endotoxaemia. The aim of this study was to find out whether bacterial translocation of Gram negative organisms (Experiment A) and endotoxaemia (Experiment B) can be prevented by selective decontamination of the digestive tract with oral tobramycin an polymyxin B, which both eliminate Gram negative rods and bind with endotoxin. Peritonitis was caused in four groups of rats with intraperitoneal injection of 200 mg zymosan suspended in paraffin. Groups A1 (n=14) and B1 (n=10) were given their drugs in their drinking water, starting seven days before the zymosan was given. Rats in groups A2 (n=19) and B2 (n=10) received no antimicrobials. In the two a groups specimens from faeces, caecum, and abdominal cavity were cultured at the time of death or after five days. In the two B groups specimens of faeces were cultured daily and blood samples were taken at 0, 24, 48, 72, and 96 hours after the zymosan had been given to measure the plasma endotoxin concentration.

Faecal and caecal cultures from all group A1 rats grew $<10^3$ cfu/g, and from all group A2 rats > 10^5 cfu/g. Positive abdominal cultures were found in 10 from group A2 and 0 from group A1 ($p<0.001$). Mortality in group A1 was significantly lower.

Faecal and caecal cultures from all group B1 rats grew $<10^3$ cfu/g, and from all group B2 rats > 10^4 cfu/g. The faecal endotoxin concentration was significantly lower in the B1 rats. The mean plasma endotoxin concentration was significantly lower in the B1 rats. The mean plasma endotoxin concentration of group B1 was significantly lower than in B2 rats at 24 and 48 hours.

Selective decontamination of the digestive tract with tobramycin and polymyxin B prevents translocation of Gram negative micro-organisms and endotoxaemia, and reduces mortality, in rats with zymosan induced peritonitis.

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BACTERIAL TRANSLOCATION AND ENDOTOXIN ABSORPTION IN ORGAN DONORS.

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The mucosa of the GI-tract provides an effective barrier to the entry of intestinal bacteria and endotoxins. As shown in animal experiments this barrier function can be lost in critical illness and thus may lead to translocation of bacteria and endotoxins, resulting in bacteraemia, endogenous infections and endotoxaemia. Since hardly any information is present about these phenomena in humans without (preexistent) GI-tract disease, we did a prospective study in organ donors because they are at risk for bacterial translocation and endotoxin absorption.
Materials and methods: Twenty-one organ donors (mean age 26, range 3-58 years) entered the study. Before surgery a bloodsample was taken from the peripheral blood for culture, endotoxin level and endotoxin inactivating activity (EIA). After laparotomy a sample of abdominal fluid was taken for culture and endotoxin level. Subsequently mesenteric lymfnodes (MLN) were sampled at the proximal jejunum, the distal jejunum and the distal ileum for culture. Before starting organ perfusion a bloodsample was taken from the portal vein for culture, endotoxin level and EIA. After removal of the organs biopsies were taken of the lung, spleen and liver, for culture and from the proximal jejunum, the distal jejunum, the distal ileum and the caecum for light and electron microscopy and culture. Cultures were considered positive as more than 10^4 cfu/gram tissue or $>10^4$ cfu/ml fluid were isolated.
Results: Positive cultures were found in the MLN of 11 donors, in the lung of 7, in the liver of 2, in the spleen of 5 and in the peripheral blood of 1 donor. All cultures of portal blood and peritoneal fluid were negative. Three times only gram negative bacteria were isolated, 19 times only gram positives and 4 times both. All but five of the isolated strains were also isolated from the small bowel. Elevated plasma endotoxin levels (>5 pg/ml) in the peripheral blood were found in 4 donors, in the portal blood in 2 donors and in peritoneal fluid in 9/17 donors. The EIA (mean±SD,μg/ml) in both peripheral (2.25±2.9) and portal blood (1.94±2.6) were significantly ($p< 0.0001$) lower as in a reference group of healthy individuals (8±1). No mucosal damage was found in the biopsies.
Conclusion: Bacterial translocation is a common feature in organ donors and is probably frequently accompanied with endotoxin absorption leading to endotoxaemia and/or decreased endotoxin inactivating capacity.

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THE CLINICAL VALIDITY OF TESTING ENDOTOXIN IN SEVERE SEPSIS/SHOCK IN ICU PATIENTS

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Early treatment, within 24 h maximally, can be desirable in persisting septic shock, associated with a high mortality. Quick and highly predicting procedures for identification of the type of infection still remain a big problem. Besides fully supportive and appropriate antibiotic therapy, today adjunctive immunotherapy with antibodies against endotoxin (HA-1A, Centoxin®) is available (Ziegler EJ et al., N Engl J Med 1991; 324: 429).
Objective: to evaluate the effectiveness of determination of endotoxin (LPS) to identify endotoxemia, as a predictor of gram-negative infection; a retrospective survey after ICU admission.
The LPS concentration in serum samples was determined using the Coatest® Endotoxin test by KabiVitrum, Sweden. The assay was modified for measuring in serum or plasma. Serum samples were diluted 10-fold with endotoxin-free water and heated at 75 - 80° Celsius for 5 minutes. After cooling to room temperature, 25 μL of the pretreated solution is brought to 37° Celsius. After 4 minutes, 25 μL LAL-solution is added to each tube and the solution is stirred. Incubation time is 30 minutes. Next, 50 μL of the S2324 chromogen substrate / buffer (1 : 1) solution is added and stirred again. After another 5 minutes the reaction is terminated by adding 50 μL 50% acetic acid. After adding 450 μL water, the extinction was measured at 405 nm immediately.
Concentrations were determined by reading them from a standard curve. The sensitivity of the assay was about 25 $pg \cdot mL^{-1}$, while the time needed for the assay was 3 h, maximally, with a capacity of 15 samples in duplo.
14 ICU patients with septic shock were included; the mortality was 43%, half of which was due to sepsis. Results: 71% showed endotoxemia (57 ± 30 $pg \cdot mL^{-1}$, range 26 - 121 $pg \cdot mL^{-1}$). Three patients received adjunctive immunotherapy with HA-1A, 100 mg, and they survived. One patient received a second dose, 3 days after the first.

In conclusion:
- LPS measurement in septic shock can be of diagnostic and therapeutic value and provides a quick tool (< 3 h) in comparison with conventional blood culture techniques, which take 24 - 48 h. These are, however, of obligate value for indentification of the infection.

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Nosocomial pneumonia prophylaxis

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UTILITY OF SELECTIVE DIGESTIVE DECONTAMINATION IN A GENERAL POPULATION OF MECHANICALLY VENTILATED PATIENTS.

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To assess the utility of selective digestive decontamination (SDD) in the prophylaxis of nosocomial respiratory tract infections in a general population of mechanically ventilated (MV) patients, we performed a prospective randomized double-blind clinical study using an association of Polymyxin E, Tobramycin and Amphotericin B topically in the oropharynx and through a nasogastric tube versus placebo. Eighty MV patients have been studied (56 males and 24 females), aged 61 ± 19 yrs (mean ± SD). Thirty-nine patients received antibiotic association and 41 received placebo. Patients received Cefotaxime (2 g/6 h) during 4 days to prevent the primary endogenous infection, except when another antibiotic treatment was necessary. The overall incidence of nosocomial pneumonia and purulent bronchitis was 24% and the mortality rate was 29%. There were not significant differences between both groups (SDD and placebo) regarding the incidence of pneumonia and purulent bronchitis (23% vs 24% respectively), the mortality rate (31% vs 27%), the duration of MV (13.5 vs 12.6 days) and the length of ICU hospitalization (15.3 vs 14.3 days). By contrast, the SDD group showed a significant lower rate of bronchial colonization by gram negative bacilli (31% vs 78%, $p< 0.00003$) and particularly by *P. aeruginosa* (23% vs 67%, $p< 0.0004$), and by *Candida* (21% vs 40%, $p<0.059$). These differences were similar in pharyngeal and in gastric colonization. Although it was not statistically significant, colonization by methicillin-resistant *S. aureus* (MRSA) was higher in the SDD group (36% vs 30% in bronchial aspirates and 33% vs 18% in pharyngeal swabs). The most frequent microorganisms causing nosocomial pneumonia in both groups were *P. aeruginosa* and MRSA. Three cases of *P. aeruginosa* pneumonia in the SDD group were exogenous and three cases in the placebo group were endogenous. MRSA pneumonias in both groups were endogenous. Our results suggest that, although SDD is useful in the prevention of pharyngeal and bronchial colonization by gram negative bacilli, this treatment does not reduce the overall incidence of nosocomial respiratory tract infections and the mortality rate in a general population of MV patients. The different pathogenesis of gram negative pneumonia in both SDD and placebo group should be confirmed in further studies.

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PROPHYLAXIS OF PNEUMONIA IN MECHANICALLY VENTILATED PATIENTS BY CONTINUOUS SUBGLOTTIC ASPIRATION.

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In the pathogenesis of endogenous ventilator-associated nosocomial pneumonia (NP), the relationship between chronic aspiration through a tracheal cuff and the development of pneumonia is well established.
The aim of this study was to evaluate the efficiency of continuous suction of subglottic secretions (SS) accumulated above the cuff in the prevention of nosocomial pneumonia in mechanically ventilated patients.
Method. This prospective study was conducted on 123 patients under mechanical ventilation (MV) for more than 72 hours. They were randomized and divided into two groups: Group I (n= 60) underwent continuous aspiration of SS and Group II (n= 63) didn't receive aspiration. Both groups were intubated with a special tracheal tube (TT)(Hi-lo-evac. Mallinckrodt) which disposes of an additional lumen in order to suction the accumulated secretions above the cuff. During the period of study, pressure of the TT cuff was checked every 4 hours in both groups. In Group I, total amount of aspirated SS was measured every day and semiquantitative culture was performed each 5 days. Protected specimen brush (PSB) and bronchoalveolar lavage (BAL) were performed in patients who fulfilled the established standard criteria for NP.
Results. Both groups were similar in terms of demographic characteristics, severity of illness (APACHE II), mortality risk and risk factors for NP. Treatment with antimicrobial agents was significantly higher in Group I (91.7%) vs Group II (68.3%) ($p<0.05$). NP developed in 26 (21.1%) of the 123 patients studied, 9 (15%) in Group I and 17 (27%) in Group II ($p= 0.159$). P. aeruginosa was the most frequent bacteria isolated in PSB and BAL. The mean (± SD) number of days until development of NP was 11.4 ± 6.7 in Group I and 6.47 ± 3.45 in Group II ($p<0.05$).
Discussion. These results indicate a tendency to reduce the risk of NP in patients with MV for more than 3 days when SS are continuously aspitrated. However, further studies will be needed to confirm the validity of this method of prophylaxis.

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