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Leukocyte Mobilization, Chemiluminescence Response, and Antioxidative Capacity of the Blood in Intestinal Ischemia and Reperfusion

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Intestinal ischemia and reperfusion elicits changes in leukocyte counts and increased production of reactive oxygen species (ROS). The purpose of this study was to investigate whether these changes were followed by and/or connected with changes in the extracellular antioxidative capacity in a rat superior mesenteric artery (SMA) occlusion/reperfusion model. The SMA was occluded for 45 min and then allowed to be reperfused. Changes of leukocyte, polymorphonuclear (PMN), and lymphocyte counts, chemiluminescence (CL) of whole blood samples as a marker of ROS production, and the total antioxidative capacity of the serum were quantified at the end of ischemia and in 1 h intervals during the postischemic period up to 4 h. The myeloperoxidase (MPO) activity in the serum and intestinal tissue samples was also determined. The MPO activity in the intestinal tissue samples was significantly elevated at the end of ischemia, and this elevation lasted for the whole postischemic period. The oxidative challenge to the body induced a fast mobilization of extracellular antioxidative mechanisms already at the end of ischemia, which was followed by a significant increase in PMN counts and whole blood CL starting at the 2nd hour after reperfusion. The increased CL activity of whole blood was attributed to the increase of the circulating PMNs. No significant changes were observed in leukocyte and lymphocyte counts. It is concluded that compensatory mechanisms of the oxidative-antioxidative balance of the body react very quickly if challenged.

Keywords: Rat, neutrophils, reactive oxygen species, myeloperoxidase

INTRODUCTION

Ischemia or severe hypoxia of large regions of intestine may occur during surgical interventions on mesenteric vessels or abdominal aorta (e.g. treatment of mesenteric infarction, reconstruction of aortic aneurysms).[1] Mesenteric vasoconstriction mediated by angiotensin, and possibly vasopressin, is one of the most common pathophysiological factors associated with non-occlusive mesenteric ischemia. The consequences of the hazards mentioned above can be infarction, sepsis, and, if left untreated, death from septic shock.[2]

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Oxidative damage to the tissues is a serious hazard in ischemia/reperfusion injury.[3] The source of the reactive oxygen species is either the ischemic/reperfused tissue itself[3,4] or the activated immune cells, especially the polymorphonuclear cells—PMNs.[5] Reactive oxygen species also take place in the recruitment of PMNs, which can lead to further activation of the immune processes.[6,7] The extent and the sequence of these events during early postischemic period play an important role in the determination of later development of organ damages, also beyond the affected vascular bed, and of circulatory shock.

We found characteristic changes in leukocyte counts and the chemiluminescence (CL) response of phagocytes during ischemia/reperfusion of the rat small intestine in our previous studies.[8] The number of leukocytes increased significantly from the early period of postischemic period. This increase was caused especially by the increase of neutrophils, while there were no substantial changes in lymphocyte counts. A highly significant increase of the total CL activity of blood samples was also observed. These results indicated that there was an increased challenge to the redox system of the body.

It is well known that a very sophisticated balance exists between prooxidants and antioxidants in the organism. If the redox system is challenged either in disease or in experimental conditions, antioxidative defence mechanisms are simultaneously stimulated.[9,10] Recent studies were focussed mainly on cellular enzymatic antioxidative systems such as superoxide dismutase, catalase, glutathione system, etc.[10] However, very little information[11] is available about compensatory mechanisms in serum, which are a good indicator of the natural defence mechanisms. Thus, in the present study the total antioxidative capacity of the serum of rats with the ischemic/reperfused small intestine was measured to ascertain whether changes in cell counts and production of phagocyte-derived reactive oxygen species were followed by and/or connected with any changes in the extracellular antioxidative status.

MATERIALS AND METHODS

Animals and Anaesthesia

Male Wistar rats were kept under controlled light and temperature conditions; water and food were given *ad libitum* before the experiments. The rats were subjected to the experimental procedure at the age of three months (250–300 g of body weight). The animals were intraperitoneally anaesthetized with ketamine/xylazine (20/2 mg per 100 g of body weight). Maintenance doses of anaesthetics were given every hour. All experiments were performed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals.

Surgical Procedures

Anaesthetized animals in the experimental group (n = 50) underwent the surgical procedure as follows. A midline laparotomy was carried out, the small intestine was exteriorized, and the root of the mesentery exposed. A thread was pulled around the superior mesenteric artery (SMA). Care was taken not to damage the nervous and lymphatic supplies of the mesentery. The thread was pulled through a polyethylene tube which was immobilized in the abdominal wound following closure of the laparotomy. Ischemia lasted for 45 minutes; then the SMA was allowed to be reperfused. The animals were kept under anaesthesia on the heated pad (the temperature was kept at 37°C) at room temperature and they were allowed to breathe spontaneously. Blood was collected at the end of the ischemic period (n = 10), 1h after reperfusion (n = 10), 2h after reperfusion (n = 10), 3h after reperfusion (n = 10) and 4h after reperfusion (n = 10) via a heart puncture. The chest was quickly opened and 5 ml blood was rapidly removed from the

right ventricle. The animals were sacrificed at individual sampling time points when blood collection was completed. Part of the collected blood (0.5 ml) was used for blood gas, hematocrit, chemiluminescence, and cell count determinations. The rest of blood was stored at room temperature for a maximum of one hour to clot blood. Serum was obtained after centrifugation (10 minutes, 1000 g), and it was stored at –30°C for myeloperoxidase (MPO) activity and total antioxidative capacity determinations. MPO activity of a 10–15 cm long small intestinal segment from the proximal jejunum was also measured.

In the sham operated group (n = 50), the identical laparotomy was carried out in anaesthetized animals, but the SMA was not occluded. All other procedures were identical to those described above in the respective groups.

In control group (n = 10), the animals were anaesthetized and immediate heart puncture for blood sampling was performed.

Chemiluminescence Assay

Luminol-enhanced chemiluminescence of phagocytes was measured using a microcomputer-controlled Luminometer 1251 (BioOrbit, Finland). The method is based on the descriptions by Lilius and Waris[12] and modified as described pre-viously.[13] The principle is based on luminol interaction with the phagocyte-derived oxidizing species, which results in large measurable amounts of light at a peak wavelength of 425 nm. The luminometer was set to measure the resulting light emission in 25 samples for a period of 65 minutes. Each sample was measured 20 times at 201-second intervals. The temperature was maintained at 37°C. The samples contained 5.0 µl blood, 50 µl 10^{-4}M luminol (Sigma, USA) in borate buffer, 50 µl opsonized zymosan (Sigma, USA) particles (0.5% in phosphate-buffered saline). The total volume of 500 µl was reached by adding Hanks balanced salt solution. This solution and borate buffer were verified so that they did not have any phagocytosis activating or inhibiting effects. The assays were run in triplicates. CL values are expressed as the integral of the obtained kinetic curves which correspond to the total amount of light produced during the time of the measurements. Spontaneous chemiluminescence without activator was also measured in all samples. This parameter corresponded to the background value and did not change in any experimental or sham operated group (data not shown).

Determination of Myeloperoxidase Activity

MPO activity was determined from segments of the proximal small intestine by a modification of the technique described by Bradley *et al.*,[14] and Fullerton *et al.*[15] Briefly: Jejunal segments were obtained from control animals and animals subjected to ischemia/reperfusion. Each segment was washed in phosphate buffered saline and homogenized in 4 ml of 20 mM phosphate-potassium buffer (pH = 7.4). After centrifugation (1000 g for 30 minutes) the sediment was resuspended in 4 ml of 50 mM phosphate-potassium buffer (pH = 6.0) containing a detergent (0.5% of hexadecyltrimethyl-ammonium bromide), and frozen for 24 hours (–70°C). After thawing, the samples were sonicated for 90 seconds and incubated for 2 hours at 60°C. After another centrifugation (10 minutes, 2000 g), a clear supernatant was achieved. Then 0.1 ml of supernatant was mixed with 2.9 ml of 50 mM phosphate-potassium buffer (pH = 6,0) containing 0.016 mg of 0-dianisidine-dihydrochloride (Sigma, USA) and 0.48 µl of 30% hydrogen peroxide. The change in absorbancy at 460 nm was measured in a spectrophotometer (Spekol 11, Carl Zeiss, Germany) at room temperature. MPO activity of the serum samples was evaluated in the same way. Serum (100 µl) was resuspended in 4 ml of 50 mM phosphate-potassium buffer (pH = 6.0) containing a detergent (0.5% of hexadecyltrimethyl-ammonium bromide), and frozen for 24 hours (–70°C). The further procedure was the same as for the tissue samples, without sonication. One unit of MPO activity was defined as the amount of enzyme reducing 1µmol peroxide/

min. The results are expressed as units per gram tissue (U/g) or units per 100µl serum (U/100µl).

CL Assay of the Total Antioxidative Capacity of the Serum

The principle of the method was described previously.[16] Peroxyl radicals produced at a constant rate by thermal decomposition of 2,2-azo-bis-2-amidinopropane hydrochloride (ABAP) are monitored by luminol-enhanced CL. The reaction was initiated by mixing 475µl phosphate buffered saline, 50µl 10^{-4}M luminol, and 50µl 400 mM ABAP. This mixture was incubated in the temperature controlled sample carousel of the luminometer (37°C) for 15 min to produce reactive radicals while light emission was measured at intervals of 3 seconds. During this period of time a steady state of the CL signal was reached. Then 20µl of serum was added directly into the cuvette and the samples were measured for another 15 min. The total antioxidative capacity of the serum was defined as the time necessary for a 50% recovery of the CL of the original steady state signal. This 50% level was chosen according to previous experiments at our laboratory (unpublished data). All other solutes used had no radical-scavenging properties. The assays were run in triplicates.

Statistical Analyses

All data are expressed as mean values ± SEM. For data analyses a program package (Statgraphics) was used. One-, two-, and multiple-way analyses of variance (ANOVA), regression analysis, and the T-test were used. The level of significance was: $p \leq 0.05$.

RESULTS

Blood gas and hematocrit values did not change during the measurements in both sham and experimental groups (data not shown). Total leukocyte counts are summarized in Figure 1a. The changes in both groups of animals did not prove to be statistically significant ($p = 0.3$ and 0.5) in the sham and occluded groups, respectively. The number of granulocytes was significantly higher at 4h of the postischemic period in the sham group when compared to the control value. There was a continuous increase of granulocyte counts in the experimental group, which started being significant at 2h of the postischemic period (Fig. 1b). The decreasing tendency in lymphocyte counts did not reach the level of significance (Fig. 1c).

Total CL of the blood samples increased both in the sham operated and in the experimental groups (Fig. 2a). There was a significant correlation between PMN counts and CL in each groups. Correlation coefficient for the experimental group was 0.54 ($p < 0.002$) and 0.82 for the sham operated group ($p < 0.0001$). The CL activity of the samples corrected for 10^3 PMNs did not change significantly either in the experimental or in the sham operated group (Fig. 2b).

The MPO activity in the proximal small intestine from control animals was 1.46 ± 0.31 U/g tissue. This basic activity did not change either at the end of ischemia or during the postischemic period in the sham operated group. On the other hand, MPO in the intestinal segments of experimental animals showed a significant increase. The results are summarized in Figure 3. MPO activity in serum was very low (0.048 ± 0.005 U/100 µl serum) and there was no change in the serum samples as a function of reperfusion time (data not shown).

The total antioxidative capacity increased in the serum samples of the animals with the occluded and reperfused SMA, while only a weak increase of the total antioxidative capacity was observed in serum samples of the sham operated group (Fig. 4). With the exception of the 2h of the postischemic period value ($p = 0.09$) the total antioxidative capacity of the experimental animals was always higher than that of the sham group during reperfusion.

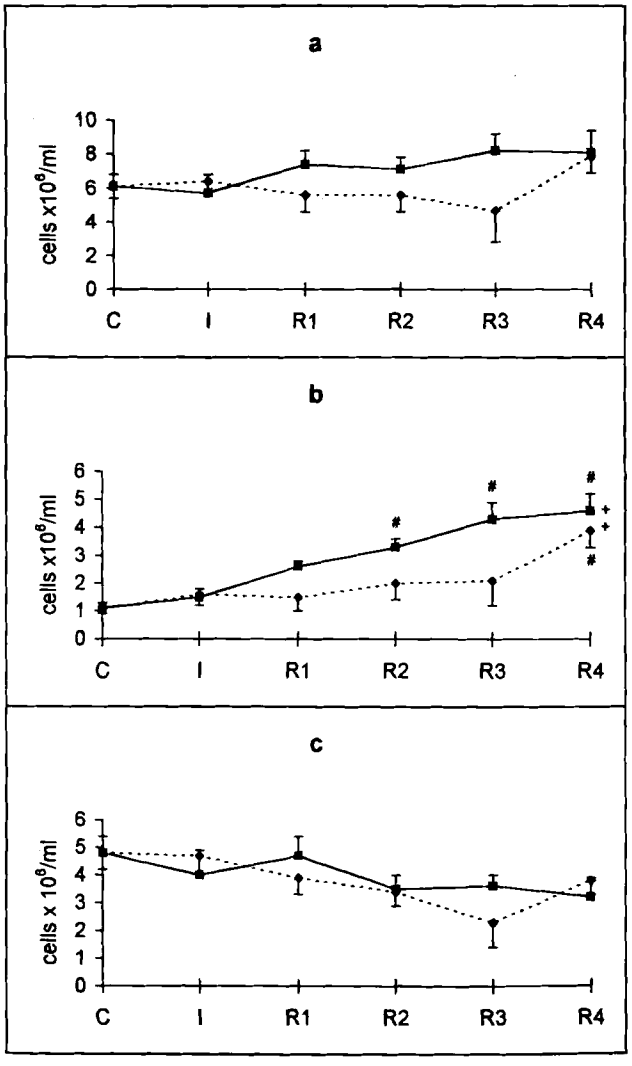

FIGURE 1 Changes in leukocytes **(a)**, neutrophils **(b)**, and lymphocytes **(c)** in rat intestinal ischemia and reperfusion. Dashed lines represent values of the sham operated group, solid lines those of the experimental groups. C ... preoperational control, I ... end of the ischemic period, R1–R4 ... 1–4 hours of postischemic period. Symbol "+" at the end of postischemic period shows the statistically significant changes of the given parameter during the followed period (ANOVA); symbol "#" shows significant difference compared to the preoperational value (ANOVA).

DISCUSSION

Here, we found an early rise in the antioxidative capacity of the plasma, which was followed by an increased number of PMNs, and there was also a simultaneous rise in the stimulated chemiluminescence of the whole blood. However, CL corrected for 10^3 PMNs, as a sign of possible PMN activation, did not change.

In our earlier studies we had a different experimental protocol.[17,18] The animals received massive fluid resuscitation during reperfusion. The sham operated controls received the same amount of crystalloid solution, and these rats were overloaded with fluid resulting in an increased cardiac output and peripheral circulation. There was no fluid resuscitation in the present series. We also reduced the surgical stress in

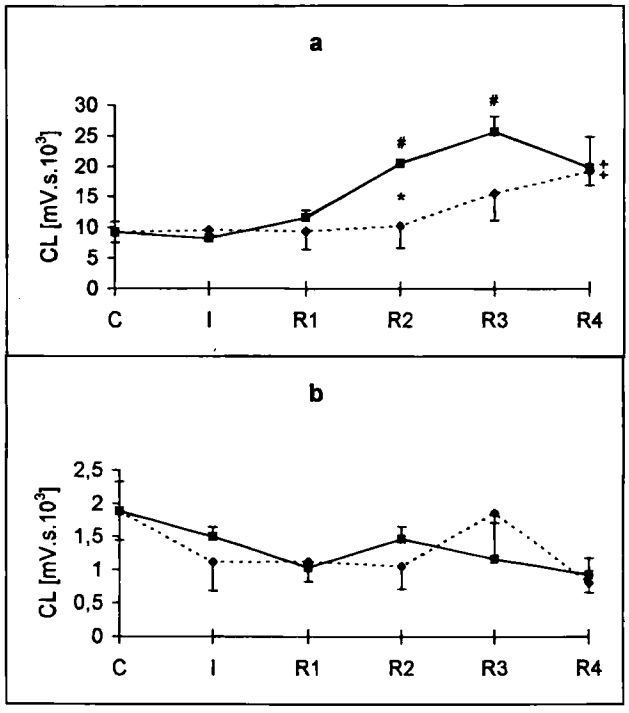

FIGURE 2 Changes of total CL **(a)** and CL corrected for 10^3 PMNs **(b)** of the whole blood in rat intestinal ischemia and reperfusion. Symbol "*" shows significant differences (t-test) between corresponding points of the two groups (sham vs. operated). Other symbols and explanations are identical to Fig. 1.

the present studies because there were no vascular cannulations compared to the earlier ones. Due to these differences we can explain why the PMN counts did not rise in the sham operated group during the first three hours of the postischemic period. However, at the end of the studies (at 4h of the postischemic period) there was a significant increase in the neutrophil count in the sham operated group, which is still unexplained. The effect of anaesthesia can be excluded since the anaesthetics used in our experiments are known to have only short-term side effects on the

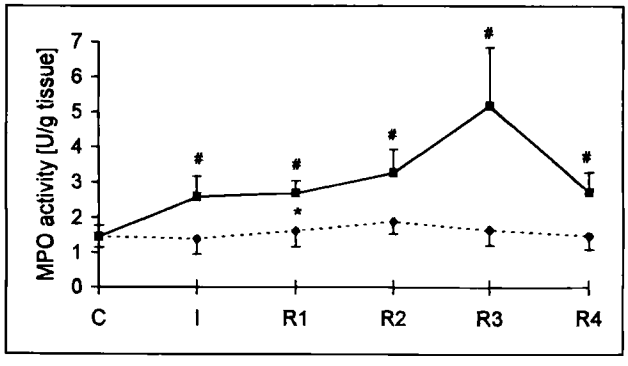

FIGURE 3 MPO activity of intestinal tissue samples in rat intestinal ischemia and reperfusion. Symbols and explanations are identical to Figs. 1 and 2.

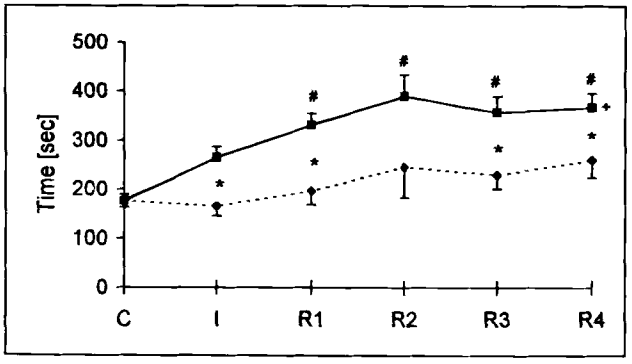

FIGURE 4 Total antioxidative capacity of the serum in rat intestinal ischemia and reperfusion. Symbols and explanations are identical to Figs. 1 and 2.

cardiovascular system. Probably, one of the explanations could be the operative trauma which is discussed below.

PMN counts started to increase in the peripheral blood, according to our earlier results, at the end of the second hour of the postischemic period. This rise corresponded with the increase of CL. The antioxidative capacity of the serum, however, started to increase already at the end of ischemia. There are several antioxidative systems in the blood[10,11,19,20] and these maintain a balance between oxidative and antioxidative mechanisms in the steady state. It is not known at the moment how this balanced mechanism behaves if one of the sides is challenged. There is no information about the way of activation of the regulatory mechanisms.

PMN infiltration as assessed by tissue MPO assay which provides an index of the number of peroxidase-positive granulocytes has been shown in numerous tissues including the inflamed rat dermis, the ischemic/reperfused dog and rabbit myocardium and the ischemic/reperfused rat intestine.[6,14,21] Despite the neutrophils are the predominant leukocytes that infiltrate the intestinal tissue following ischemia/reperfusion,[22] MPO assay is not specific for myeloperoxidase and could also measure other peroxidases such as eosinophil peroxidase or contaminating hemoglobin. Contribution of the eosinophil peroxidase to the peroxidase activity measured in our experiments could be excluded since the eosinophil number was very low not exceeding 2% of total leukocytes in any group. The contamination of intestinal tissue samples with hemoglobin after the preparation procedure could be neglected. Moreover, in serum samples, where the contamination with hemoglobin was obviously higher, the total peroxidase activity was very low indicating the minor role of hemoglobin. Taking together, the peroxidase activity measured in our experiments was due to the myeloperoxidase. The tissue concentration of MPO increased already at the end of ischemia. This result can contradict our earlier findings in which inflammatory cell accumulation in the intestinal mucosa was found only during the third hour of the postischemic period.[17] The discrepancy can be attributed to some methodological differences because intestinal segments for MPO determination were harvested from the proximal jejunum, a part of the gut that was not completely ischemic; however, segments of the distal jejunum were analyzed histologically earlier. MPO activity did not rise in the serum of the experimental group indicating that, although there was an increase in the PMN count, the enzyme did not spill into serum due to possible activation of the granulocytes.

The present model does not consist of complete intestinal ischemia.[23,24] The upper and

lower parts of the small gut do have some blood supply during SMA occlusion. There is a severe hypoperfusion and a consequent hypoxia in these segments. It is assumed that these hypoperfused areas can give signals to redox systems already during the ischemic period. This assumption can be supported by the results of Weixiong *et al.*[25] and Grotz *et al.*[26] Weixiong and co-workers used spin trapping to follow the history of free radical production and found a very early release of these substances. Grotz and colleagues in an experimental model similar to ours found an increase of TNFα concentration in the blood already at the end of 45 min of ischemia. They also found a similar rise of this cytokine (which plays the central role in initiating the inflammatory cascade) in the sham operated group; however, the rise in the experimental group was higher. They thought that the operative trauma due to laparotomy was responsible for the increase in the sham operated group. Similarly, Wanner *et al.*[27] assessed increased serum levels of proinflammatory cytokine IL-6 in sham operated rats undergoing only midline laparatomy.

The most important and original result of our studies is the finding that the natural antioxidative mechanisms of the body are mobilized already at the end of 45min-ischemia of rat small intestine due to the oxidative challenge and that their mobilization lasts at least for 4 hours after reperfusion. The chemiluminescence method used in our experiments detects the activity of chain-breaking antioxidants in serum[16] which means that the increased total antioxidant activity measured in our experimental model is related to the protection from lipid peroxidation. In our preliminary study when the serum concentrations of uric acid, vitamin E, ascorbic acid, albumin and bilirubin were measured, a good correlation ($r = 0.86$) between total antioxidant capacity of serum and serum concentration of uric acid was found. Concentrations of other antioxidants did not change significantly. Nevertheless, a contribution of individual antioxidants to the total antioxidative capacity, a time which are the antioxidants mobilized for before they are exhausted, as well as initiating signals and pathways leading to their mobilization still await detailed evaluation. Elucidation of these questions should result in novel therapeutic antioxidant strategies based on the modulation of natural endogenous antioxidants beside the commonly used therapy based on the supply of exogenous antioxidants.

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References

[1] Lehmann, C., Luther, B., Holzapfel, A., Roth, S., David, H., Grune, T., Siems, W., Burger K. and Kox, W. J. (1995). Perioperative vascular flushing perfusion in acute mesenteric artery occlusion. *European Journal of Vascular and Endovascular Surgery*, **10**, 265–271.

[2] Wilcox, M. G., Howard, T. J., Plaskon, L. A., Unthank, J. L. and Madura, J. A. (1995). Current theories of pathogenesis and treatment of nonocclusive mesenteric ischemia. *Digestive Diseases and Sciences*, **40**, 709–716.

[3] Granger, D. N., Rutili, G. and McCord, J. M. (1981). Superoxide radicals in feline intestinal ischemia. *Gastroenterology*, **81**, 22–29.

[4] Haglund, U. (1991). Hypoxic damage of the gut in shock. In *Shock, Sepsis, and Organ Failure* (eds. G. Schlag andH. Redl), Springer Verlag, Berlin, Heidelberg, New York, London, Paris, Hong Kong, Barcelona, Budapest, pp. 3–22.

[5] Granger, D. N. (1988). Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *American Journal of Physiology*, **255**, H1269–H1275.

[6] Grisham, M. B., Hernandez, L. A. and Granger, D. N. (1986). Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *American Journal of Physiology*, **251**, G567–574.

[7] Otamiri, T. (1989). Oxygen radicals, lipid peroxidation, and neutrophil infiltration after small-intestinal ischemia and reperfusion. *Surgery*, **105**, 593–597.

[8] M. Číž, A. Lojek, M. Feilhauerová, H. Slavíková and J. Vondráček (1995). Metabolic activity of rat peripheral phagocytes during ischemia and reperfusion of small intestine. *Shock*, **3**, Suppl. 1, 19.

[9] Jenkins, R. R., Kohman, L. J. and Veit, L. J. (1994). The relationship of iron and glycogen to the in vitro, ultraweak chemiluminescent analysis of lipid peroxidation in rabbit hearts of varying ages. *Free Radical Biology and Medicine*, **16**, 627–631.

[10] Urban, T., Hurbain, I., Urban, M., Clement, A. and Housset, B. (1995). Oxidants and antioxidants. Biological effects and therapeutic perspectives. *Annales de Chirurgie*, **49**, 427–434.

[11] Das, D. K. and Maulik, N. (1994). Antioxidant effectiveness in ischemia-reperfusion tissue injury. *Methods in Enzymology*, **233**, 601–610.

[12] Lilius, E. M. and Waris, M. (1984). A very sensitive and rapid chemiluminescence method for the measurement of phagocytosis. In *Analytical applications of bioluminescence and chemiluminescence* (eds. L. J. Kricka, P. E. Stanley, G. H. G. Thorpe and T. P. Whitehead), Academic Press, London, pp. 401–404.

[13] Číž, M. and Lojek, A. (1993). Kinetics of luminol enhanced chemiluminescence induced in murine splenocytes and bone marrow cells by various stimulating agents. *Folia Biologica (Prague)*, **39**, 106–116.

[14] Bradley, P. P., Priebad, D. A. and Christensen, R. D. (1982). Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. *Journal of Investigative Dermatology*, **78**, 206–209.

[15] Fullerton, D. A., Hahn, A., Koike, K., Banerjee, A. and Harken, A. H. (1993). Intracellular mechanism of pulmonary vasomotor dysfunction in acute lung injury caused by mesenteric ischemia-reperfusion. *Surgery*, **114**, 360–367.

[16] Uotila, J. T., Kirkkola, A. L., Rorarius, M., Tuimala, R. J. and Metsä-Ketelä, T. (1994). The total peroxyl radical trapping ability of plasma and cerebrospinal fluid in normal and preeclamptic partiturients. *Free Radical Biology and Medicine*, **16**, 581–590.

[17] Illyés, G. and Hamar, J. (1992). Sequence of morphological alterations in a small intestinal ischaemia-reperfusion model of the anesthetized rat. A light microscopic study. *International Journal of Experimental Pathology*, **73**, 161–172.

[18] Hamar, J., Dézsi, L., Ádám, É., Egri, L., Netzer, K. O., Stark, M. and Lutz, J. (1987). Role of fluid replacement, increased oxygen availability by perfluorochemicals, and enhanced RES function in the treatment of mesenteric occlusion shock. *Research in Experimental Medicine*, **187**, 451–459.

[19] Soriani, M., Pietraforte D. and Minetti, M. (1994). Antioxidant potential of anaerobic human plasma: role of serum albumin and thiols as scavengers of carbon radicals. *Archives of Biochemistry and Biophysics*, **312**, 80–88.

[20] Wayner, D. D. M., Burton, G. W., Ingold, K. U., Barclay, L. R. C. and Locke, S. J. (1987). The relative contributions of vitamin E, urate, ascorbate, and proteins to the peroxyle radical-trapping antioxidant activity of human blood plasma. *Biochimica et Biophysica Acta*, **924**, 408–419.

[21] Laight, D. W., Lad, N., Woodward, B. and Waterfall, J. F. (1994). Assessment of myeloperoxidase activity in renal tissue after ischaemia/reperfusion. *European Journal of* Pharmacology—Environmental Toxicology and Pharmacology Section, **292**, 81–81.

[22] Oliver, M. G., Specian, R. D., Perry, M. A. and Granger, D. N. (1991). Morphologic assessment of leukocyte-endothelial cell interaction in mesenteric venules subjected to ischemia and reperfusion. *Inflammation*, **15**, 331–346.

[23] Megison, S. M., Horton, J. H., Chao, H. E. and Walker, P. B. (1990). A new model for intestinal ischemia in the rat. *Journal of Surgical Research*, **49**, 168–173.

[24] Horton, J. H. (1992). Alterations in intestinal permeability and blood flow in a new model of mesenteric ischemia. *Circulatory Shock*, **36**, 134–139.

[25] Weixiong, H., Aneman, A., Nilsson U. and Lundgren, O. (1994). Quantification of tissue damage in the feline small intestine during ischemia-reperfusion: the importance of free radicals. *Acta Physiologica Scandinavcica*, **150**, 241–250.

[26] Grotz, M. R. W., Ding, J., Gou, W., Huang, Q. and Deitch, E. A. (1995). Comparison of plasma cytokine levels in rats subjected to superior mesenteric artery occlusion or hemorrhagic shock. *Shock*, **3**, 362–368.

[27] Wanner, G. A., Ertel, W., Müller, P., Höfer, Y., Leiderer, R., Menger, M. D. and Messmer, K. (1996). Liver ischemia and reperfusion induces a systemic inflammatory response through Kupffer cell activation. *Shock*, **5**, 34–40