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# MORPHOLOGICAL AND RADIOMETRIC EVALUATION OF INTRAVASCULAR COAGULATION IN EXPERIMENTAL BURN AND ENDOTOXIC SHOCK

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*The authors performed comparative morphological and radiometric (with $^{131}$J-fibrinogen) study of the severity and dynamics of intravascular coagulation (IC) during burn and endotoxic shock in white male Wistar rats. An early (at the 15$^{th}$ min) high intensity of IC was established in lungs and adrenals but a late peak of IC (until the 24$^{th}$ hour) was found out in kidneys, intestine, liver and spleen. IC intensity reduced significantly at the end of the first hour. There was some discrepancy between $^{131}$J-fibrinogen accumulation and density of microthrombi in different organs.*

**Key-words**: Intravascular coagulation, burn shock, endotoxic shock, $^{131}$J-fibrinogen, pathomorphology, rats

## INTRODUCTION

The density and organ distribution rate of microthrombi as a morphological manifestation of intravascular coagulation (IC) vary considerably depending on the degree and speed of thrombolysis. That is why the estimation of IC severity based on histologic examination is not always sufficient (2, 14). Only in a few experiments attempts have been made to determine the value of the morphological method for IC severity assessment (23, 25, 27).

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The purpose of the present study is to evaluate comparatively the possibilities of both morphological and radiometric methods for IC severity estimation in experimental models of burn and endotoxic shock.

## MATERIAL AND METHODS

Adult white male Wistar rats (weight of 200-220 g) divided into three groups were used: Group one - 57 animals with burn shock caused by skin burn of 3$^{rd}$b degree of 15 % of the body surface with a radiant heat device; Group two - 43 animals with endotoxic shock induced by twofold (with an interval of 24 hours) intravenous application of a lipopolysaccharide from *Escherichia coli*

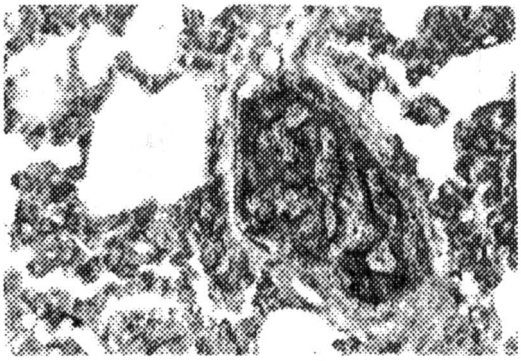

*Fig. 1. Mixed microthrombi in muscular pulmonary artery - burn shock. Staining with azan-Cruchay. Magn. 10 x 20*

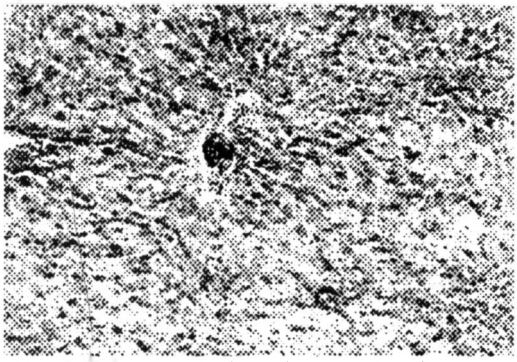

*Fig. 2. Fibrin microthrombi in a portal vein branch - endotoxic shock. Staining picromallory-Caarstears. Magn. 10 x 6,3*

$O_{111}$ in a dose of 5 mg/kg b. w. for the first and of 15 mg/kg b. w. for the second injection; Group three - 115 control rats. All test and control animals were i. v. given 10 μCi $^{131}$J-human fibrinogen (AB Kabi Stockholm, Sweden). Rats were sacrified under light aether narcosis 15 min, 1, 2 and 24 hours after the beginning of the experiment. Autopsy was carried out immediately after death. Pieces of brain, lungs, heart, liver, kidneys, spleen, intestine, adrenals and striated muscle were taken for morphological and radiometric examination. Paraffin sections were stained with HE, PAS, phosphotungistic acid hematoxylin (PTAH), azan - Cruchay and picromallory - Caarstears. Pieces for radiometric measurement were weighed with analytical balance. Additionally, 1 ml of blood was taken from each animal for radiometry.

Small pieces (about 1 mm$^3$ in size) from lungs, kidneys, myocardium, and liver were fixed in 5 % glutaraldehyde, postfixed in 1 % osmium tetraoxide and embedded in Durcupan ACM (Fluka). Ultrathin sections were examined on transmission electron microscope JEM 7A and Opton EM 109 Turbo. Radioactivity was measured in a shielded-wall scintillation detector with sodium iodine crystal. Tissue sample radioactivity was determined in counts per 1 g of tissue/min. Blood reactivity of each animal was set to 100 and all other data were related to this. The statistical evaluation of the differences between the groups was carried out with alternative and variation analysis at a confidence threshold of $p < 0.05$.

## RESULTS

Early development of IC with formation of microthrombi in internal organs in all the animals of the burn and endotoxic shock groups was established (Fig. 1 and Fig. 2). Distribution rate of microthrombi in various organs was significantly higher in endotoxic shock (in 29,63 ± 3,65 % of the cases) than that in burn one (in 17,90 ± 2,23 % of the cases) ($p < 0.001$). Dynamic follow-up of IC distribution rate demonstrated histological and radiometric evidence of its decrease in the first hour and another increase during the second hour. Besides microthrombus

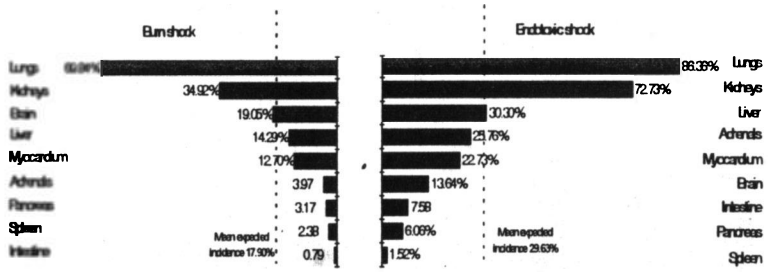


*Fig. 3. Organ predilection of IC in burn and endotoxic shock*

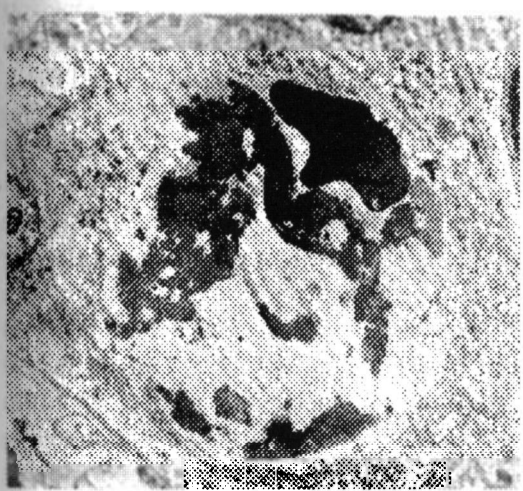

*Fig. 4. Fibrin particles in glomerular capillary - endotoxic shock. Magn. x 4000*

number continued to increase up to the 24th hour in endotoxic shock while it decreased again and reached its lowest values at the 24th hour in burn shock.

Morphological investigation revealed that lungs and kidneys (Fig. 3) were the organs most commonly affected in both experimental models. Ultrastructurally visible fibrin strands and lumps in most capillaries of the lungs, kidneys, and myocardium were an important finding (Fig. 4). The comparison of morphological data with $^{131}$J-fibrinogen/fibrin deposition revealed irregular IC intensity with a distinct time-dependent pattern in the single organ. Early deposition in lungs and adrenals (in the initial 15 min) and a later peak and more delayed fall (untill the 24th hour) of IC activity in kidneys and liver were observed. Radiometric data from lungs and kidneys corresponded very closely to morphological results. Thus the highest $^{131}$J-fibrinogen/fibrin deposition was at the 15th min and on the 24th hour for the lungs (Fig. 5) but at the 15th min only for the kidneys (Fig. 6). Fibrin deposition was significantly higher in both mentioned organs in the endotoxic shock group ($p < 0.001$). The highest $^{131}$J-fibrinogen deposition in adrenals was found out at the 15th min after the onset of endotoxic shock. Histologically, however, only a few microthrombi were established

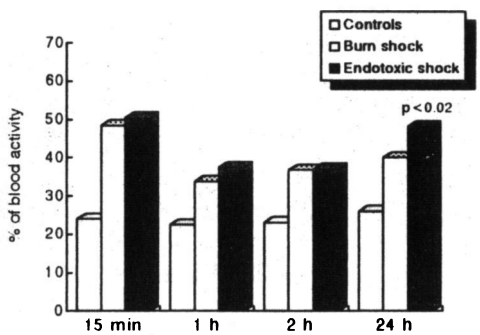


*Fig. 5. Dynamics of $^{131}$J-fibrinogen accumulation in lungs during shock-induced IC*

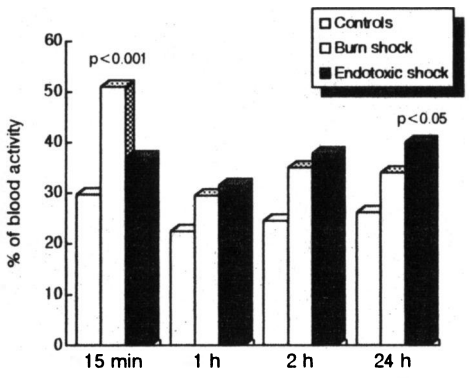


*Fig. 6. Dynamics of $^{131}$J-fibrinogen accumulation in kidneys during shock-induced IC*

at the same time.

More delayed and gradually increasing $^{131}$J-fibrinogen deposition was observed in both liver and spleen. It reached its maximal values at the 24$^{th}$ hour being more outlined in endotoxic shock. There was discrepancy between the degree of radioactivity and the density of morphologically proved microthrombi in these organs. At the same time, PAS-positive deposits in the cytoplasm of stellate reticuloendothelial cells (K u p f e r ' s cells) were seen. Probably, they resulted in fibrin degradation product (FDP) phagocytosis. Similar data were obtained for the intestine, too. The slight and insignificant increase of the myocardial and cerebral radioactivity corresponded to the low density of histologically proved microthrombi in these organs.

## DISCUSSION

The comparative analysis of the data obtained by both methods allows a better understanding and quantitative determination of IC dynamics in various organs. It is established that lungs, kidneys (for burn shock), and adrenals (for endotoxic shock) are early target organs. Brain (for burn shock) and intestine (for endotoxic shock) are considered subsequent target organs. The delayed maximal activity (at the 24$^{th}$ hour) in the liver and spleen as compared to that in other organs might indicate that at least some part of this activity in the liver and spleen is not due to fibrin deposition (or to microthrombi, respectively) but rather to accumulation of fibrin monomers and FDP. These results demonstrate a certain discrepancy between the morphological evidence of microthrombi and $^{131}$J-fibrinogen/fibrin deposition in single organs. There exist several possibilities for it: 1. Early activation of fibrinolysis with disappearance of some microthrombi (3, 9) with accumulation of fibrin-monomers and FDP in the blood flow; 2. Low fibrin content of the microthrombi containing chiefly platelets or erythrocytes; 3. During the radiometric determination not only completely polymerized labeled fibrin but also some intermediate products such as fibrin-monomers, partially polymerized low-molecular fibrin, FDP, and small quantities of unchanged labeled fibrinogen can be recorded. However, all these derivatives of fibrinogen transformation remain morphologically invisible.

This discrepancy is probably due to FDP accumulation in the cells of the monocyte-macrophageal system when liver and spleen both are concerned. The role of the latter for the elimination of FDP and other coagulation products is well-known (10, 11, 27). An incomplete IC without final fibrin polymerization (6, 13, 22, 26) or predominant "ultramicrothrombus"

formation (21) can be supposed for the kidneys and intestine. It has been electron-microscopically confirmed in the present study.

The results from different experimental models of IC indicate that organ distribution of fibrin and accumulation of labeled fibrinogen, respectively, depends on the route of application and kind of procoagulant stimulus. That is why the data reported by single investigators are contradictory (12, 16, 18, 23 - 25, 27). This fact has to be taken into consideration when comparing and evaluating results from various experimental studies of IC.

## CONSLUSION

Our analysis suggests the great importance of the concept of IC as a process of transient coagulation occurring in the blood flow for its morphological evaluation. Fibrin monomers formed during this process reach different stages of polymerization. Obviously, the so-called "ultramicrothrombi" present the first morphologically detectable product of IC. Formation of the larger microthrombi visible by conventional microscopy is a non-obligatory end result of IC. An incomplete fibrin polymerization or an early fibrinolysis activation leads to a less expressed thrombotic obstruction of the microcirculatory bed.

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## Morphologische und radiometrische Einschätzung der intravasalen Gerinnung beim experimentellen Verbrennungsschock und Endotoxinschock

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**Zusammenfassung**: Es wurde eine vergleichende morphologische und radiometrische Untersuchung (mit $^{131}$J-Fibrinogen) der Schwere und der Dynamik von der intravasalen Gerinnung (IG) bei männlichen weißen Wistar Ratten während des Verbrennungsschock und des Endotoxinschocks durchgeführt. Eine frühe (an der 15. Minute) hohe Intensität der IG in den Lungen und den Nebennieren, wie auch eine späte (bis zur 24. Stunde) hohe Intensität der IG in den Nieren, dem Dünndarm, der Leber und der Milz wurden festgestellt. Diese Intensität nahm bedeutend am Ende der ersten Stunde ab. Es gab einen gewißen Unterschied zwischen der Akkumulierung des $^{131}$J-Fibrinogens und der Dichte der Mikrothromben in den verschiedenen Organen.

## Évaluation morphologique et radiométrique de la coagulation intravasculaire au cours des chocs thermique et endotoxique

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**Résumé**: On a réalisé une analyse comparative morphologique et radiométrique (avec $^{131}$J-fibrinogène) de la gravité et de la dynamique de la coagulation intravasculaire (CIV) au cours d'un choc thermique et d'un choc endotoxique. On a expérimenté sur des rats mâles blancs Wistar. À la 15$^{ème}$ minute l'intensité de la CIV augmente dans les poumons et les glandes surrénales et plus tard (a la fin du 24$^{ème}$ heure) - dans les reins, l'intestin grêle, le foie et le rate. L'intensité de la

CIV diminue considérablement à la fin de la première heure. On constate une certaine disproportion entre la quantité accumulée de $^{131}$J-fibrinogène et la densité des microthrombi dans les organes différents.