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Quantitative Determination of Fibrin Deposition in Organs of Rats in the Early Post-burn Period*

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Summary. Rats injected with labelled fibrinogen were subjected to a standardized, third degree *thermal injury* and sacrificed at various intervals after the burn for radioactivity measurement of blood and organ samples. 5 min post-burn a moderate elevation of the lung radioactivity was found, and this elevation could be prevented by previous heparinization, indicating that the increase was a result of *fibrin deposition in the lungs.*

Zusammenfassung. Es wurden Ratten einer drittgradigen Verbrühung ausgesetzt, nachdem ihnen radioaktives Fibrinogen eingespritzt wurde. Nach der Tötung in unterschiedlichen Zeitabständen von der Applikation des Trauma wurde die Radioaktivität in Organen und Blut gemessen. 5 min nach der Verbrühung wurde eine mäßige Erhöhung der Lungenradioaktivität festgestellt, welche durch eine Applikation von Heparin verhindert werden konnte. Diese Feststellung spricht für eine Ansammlung von Fibrin in den Lungen.

Key words: Fibrin deposition, in organs of rats — Thermal injury, fibrin deposition in the lungs.

Depressed levels of coagulation factors have been reported after burns in humans (Glants *et al.*, 1969; Novak *et al.*, 1969; Curreri *et al.*, 1970), dogs (Campbell *et al.*, 1950) and rabbits (Johansson, 1964; Hey *et al.*, 1969). In the two investigations in rabbits the decrease was prevented by previous heparinization, indicating consumption of the coagulation factors in a coaqulation process. In burned rats slight depression of coagulation factors has been observed (Arturson and Wallenius, 1964; Marggraf *et al.*, 1970; Rammer, 1972) but as heparinization was found not to influence the platelets or the fibrinogen level (Rammer, 1972) evidence of intravascular coagulation was not obtained.

Radioactivity measurement of tissue samples from experimental animals injected with isotope labelled fibrinogen offers a possibility of quantitative assessment of fibrin deposition in various organs (Saldeen, 1966, 1969; Busch *et al.*, 1972). In the present study this method was applied since it was assumed that fibrin deposition might be detectable in internal organs of burned rats even if it was not of sufficient magnitude to be revealed by changes of the plasma fibrinogen level.

Material and Methods

Experimental Animals. 75 female Sprague-Dawley rats (Anticimex Farm, Stockholm, Sweden), weighing 200 ± 5 g, were used. The animals were allowed free access to tap water and food (Ewos rat pellets).

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Labelled Fibrinogen. Human fibrinogen was labelled with ^{125}I as described elsewhere (Busch *et al.*, 1972). 48 hrs before the experiment 0.25 ml/100 g body weight of a solution containing about 0.8 mg protein and 4 μCi/ml was injected into a tail vein under ether anaesthesia.

Thermal Injury. The method has been described in detail elsewhere (Rammer, 1972). The rats were immersed in water at 90°C for 30 sec, giving a third degree scald injury, covering 14% of the body surface. The 24-hour mortality was about 13%.

Heparin. Heparin without preservative (Vitrum) in a dose of 200 IU/100 g body weight in 0.4 ml of saline was injected into a tail vein under ether anaesthesia 15 min before the burn. The coagulation time of blood collected from the carotid artery over a period of 4 hrs exceeded 30 min in normal and burned rats.

Tranexamic Acid. AMCA (AB Kabi) in a dose of 10 mg/100 g body weight in 0.4 ml of saline was injected into a tail vein under ether anaesthesia 15 min before the burn. This dose was found to prevent the post-burn increase in plasma fibrinolytic activity (Rammer, 1972).

Radioactivity Measurements of Blood and Organ Samples. Under ether anaesthesia the abdomen was opened and an 18-gauge siliconized needle was introduced into the aorta at the bifurcation. About 1 ml of blood was collected into a plastic Ellermann tube for radioactivity measurement and for triple microhaematocrit determination. One lung, one kidney and pieces of the liver and burned skin were quickly removed, dissected free from connective tissue, cleaned with filter paper and put into weighed Ellermann tubes. After weighing, the radioactivity of blood and organ samples was measured in a gammaspectrometer (Picker Nuclear, Autowell II). More than 3000 counts were always made and the samples contained more than 10 times background radioactivity.

Morphological Examination. Organ pieces were fixed in 10% neutral formalin. 5 μ thick paraffin sections were stained with Mallory's PTAH method for the demonstration of fibrin.

Calculations. The radioactivity of plasma was calculated as the ratio of ^{125}I in blood/l-haematocrit. Conventional statistical methods according to Snedecor (1965) were used. Differences between groups of animals were tested at the 5% level, using Student's *t*-test.

Experiments and Results

Experiment I. The time course of changes in organ radioactivity after the burn was studied in this experiment. Tranexamic acid was injected before the burn to prevent the error of extravascular accumulation of labelled fibrin degradation products.

In rats previously injected with labelled fibrinogen tranexamic acid was injected 15 min before the burn, and groups of 6—8 rats were killed for radioactivity measurements 5, 10, 15, 30, 60 and 240 min post-burn. Control rats were untreated.

The results of the experiment are shown in Table 1. An increase in radioactivity was found in the lung (about 40%) and in the burned skin (about 100%) 5 min post-burn but no significant elevations in the liver and kidney. The haematocrit increased from 45 to 54% in 5 min. In PTAH stained sections small amounts of material resembling fibrin were seen in lung arterioles at the 5 min interval (Fig. 1) and during the 1st hr post-burn. In the burned skin small vessels from 5 min onwards occasionally were found to contain similar material.

Experiment II. To determine whether the elevation of radioactivity in the lungs and burned skin 5 min after the burn was a result of fibrin deposition, animals pretreated with heparin and with saline were compared at that interval.

The rats were previously injected with labelled fibrinogen. 10 rats were injected with heparin and 11 rats received an equivalent amount of saline before the burn. 8 rats were injected with saline and immersed in water at 38°C. All rats were also pretreated with tranexamic acid. 5 min post-burn the animals were killed for radioactivity measurement.

Table 1. Radioactivity (cpm $\times 10^3$/g) in blood and organs at various intervals post-burn. Mean $\pm$ S.D.

	Minutes after burn						
	0	5	10	15	30	60	240
	n						
	7	6	6	8	7	6	6
Blood	13.89 ± 1.22	14.67 ± 1.56	13.23 ± 1.56	13.38 ± 2.08	12.45 ± 1.19	12.07 ± 1.58	11.09 ± 1.23[a]
haematocrit	45.1 ± 0.9	54.2 ± 3.4[a]	55.4 ± 2.9[a]	55.6 ± 2.8[a]	55.0 ± 3.3[a]	58.3 ± 2.5[a]	56.9 ± 1.7[a]
plasma	25.32 ± 2.35	32.28 ± 5.00	29.76 ± 3.34	30.22 ± 5.19	27.82 ± 3.06	29.04 ± 3.57	25.86 ± 3.42
Lung	5.48 ± 0.56	7.80 ± 0.97[a]	6.75 ± 0.87	6.88 ± 1.37	6.25 ± 0.65	6.10 ± 0.83	5.39 ± 0.77
Liver	2.22 ± 0.08	2.64 ± 0.67	2.48 ± 0.38	2.37 ± 0.52	2.19 ± 0.14	2.12 ± 0.23	2.02 ± 0.23
Kidney	3.12 ± 0.23	3.70 ± 0.76	3.23 ± 0.42	3.39 ± 0.55	2.90 ± 0.34	2.85 ± 0.36	2.30 ± 0.17
Burned skin	2.44 ± 0.39	4.92 ± 0.97[a]	5.00 ± 1.14[a]	4.37 ± 1.06[a]	4.76 ± 0.50[a]	5.24 ± 1.13[a]	4.54 ± 0.98[a]

[a] Significantly different from preburn value ($p < 0.05$).

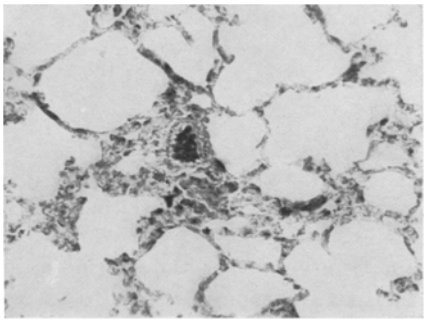

Fig. 1. PTAH stained lung section showing amorphous, darkly blue material in pulmonary arteriole (approx. × 300)

Table 2. Radioactivity (cpm × 10^3/g) in blood and organs 5 min post-burn. Mean ± S.D.

Treatment	Saline + AMCA + 38°C	Saline + AMCA + 90°C	Heparin + AMCA + 90°C
	n		
	8	11	10
Blood	16.85 ± 1.80	14.91 ± 1.39	15.54 ± 1.56
haematocrit	45.9 ± 2.4	52.1 ± 2.5[a]	52.9 ± 1.3[a]
plasma	31.24 ± 3.93	30.65 ± 3.15	31.90 ± 3.34
Lung	6.85 ± 0.75	7.25 ± 0.57	6.92 ± 0.73
lung/plasma	0.221 ± 0.008	0.239 ± 0.019[a]	0.218 ± 0.011[b]
Liver	2.59 ± 0.36	2.46 ± 0.20	2.69 ± 0.30
liver/plasma	0.084 ± 0.005	0.082 ± 0.008	0.086 ± 0.006
Kidney	3.54 ± 0.66	3.79 ± 0.44	3.86 ± 0.46
kidney/plasma	0.114 ± 0.011	0.126 ± 0.018	0.122 ± 0.006
Burned skin	2.52 ± 0.90	5.35 ± 1.12[a]	4.29 ± 0.67[a]
burned skin/plasma	0.082 ± 0.031	0.176 ± 0.037[a]	0.150 ± 0.021[a]

[a] Significantly different from value in sham-burned rats ($p < 0.05$).
[b] Significantly different from saline pretreated burned rats.

Table 2 shows the results of the experiment. Heparin pretreated animals showed a lower lung radioactivity than those pretreated with saline. The difference in lung/plasma ratio between the two groups was statistically significant.

A difference was also found in the burned skin between saline and heparin pretreated animals, but the difference was not significant either as absolute radioactivity or as tissue/plasma ratio. In the liver and kidney pretreatment with heparin had no influence on the results.

Discussion

In this study fibrin deposition in different organs of burned rats previously injected with ^{125}I-fibrinogen was measured as the difference in organ radioactivity between animals pretreated with heparin and with saline. The fibrinolysis was inhibited by previous injection of tranexamic acid to prevent the formation of

fibrin degradation products during the post-burn increase in fibrinolytic activity (Rammer, 1972). Synthetic fibrinolysis inhibitors have been found to enhance (Beller *et al.*, 1967) but not to initiate intravascular coagulation (Gajewski and Alexander, 1963). No attempt was made to quantitate the plasma and extravascular radioactivities by injection of ^{131}I albumin for calculation of the fibrin content (Busch *et al.*, 1972), as plasma volume determination in organs by means of labelled albumin was considered unreliable owing to the rapid increase in haematocrit immediately after the burn. The method for isolation of fibrin by homogenization and centrifugation of the tissue (Busch and Rammer, 1972) had been found unsuitable for studies of minute amounts of fibrin.

An increase in radioactivity was found in the lungs shortly after the burn. Pretreatment with heparin prevented this elevation, and in PTAH stained sections fibrin like material was seen in pulmonary arterioles and the increase therefore apparently was a result of intravascular fibrin deposition in the lungs. The amount of fibrin was, however, small as the difference in lung radioactivity between heparin and saline pretreated animals corresponded to only about 0.04 mg of fibrin per gram tissue, as calculated from the estimated relative specific radioactivity of plasma fibrinogen in control rats. This is a small coagulation compared to the 10 mg of fibrin per gram found in the lungs after a heavy thrombin infusion (Busch *et al.*, 1972), and it is just at the limit possible to detect with this method.

In the burned skin an increase in radioactivity was also found immediately after the burn. In the heparinized rats the mean radioactivity was about 40% lower than in the animals pretreated with saline but owing to the large variation of the results the difference was not statistically significant. In small vessels of the burned skin occasional deposits of PTAH positive fibrin like material were seen from 5 min onwards, which probably represented thrombi.

The occurrence of fibrin deposition in internal organs during subsequent days post-burn could not be determined with the present method. Continuous heparin administration tended to aggravate the condition of the burned rats, possibly owing to unlimited perfusion and plasma losses in the burned skin. Interpretation of the organ radioactivity alone was considered unreliable owing to the reported increase in the extravascular fibrinogen pool in burned patients (Davies *et al.*, 1965). Morphological examination of PTAH stained, formalin fixed material did not, however, reveal any fibrin in internal organs at later intervals after the burn.

The fibrin deposition in the lungs might be due to release of thromboplastic material from the burned skin. In addition thermal injury to the formed elements of the blood or possibly to coagulation factors might contribute to intravascular coagulation. The localization of fibrin to the lungs may be explained by their function as a filter organ for the venous return of blood.

In the burned rats of this study, and also after femoral fractures in rats (Saldeen, 1969), fibrin deposition in the lungs occurred within minutes after the injury. Intravascular fibrin deposition in the lungs is a common autopsy finding in patients dying a few days after burns and other types of trauma (Saldeen, 1964, 1967, 1970; Eeles and Sevitt, 1967), indicating that a continuous deposition of fibrin takes place in the pulmonary vessels in these patients. This deposition has been supposed to be of importance in the development of the respiratory failure often seen in patients with severe injuries (Saldeen, 1964).

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