The Abscence of Intravascular Clotting in Rat Anaphylaxis

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Summary. Intravascular fibrin formation could not be detected in various phase of IgE mediated anaphylactic shock of rats, either by using an isotope technique or testing the plasma samples by the ethanol gelation test.

Anaphylactic shock, a reaction which developes following the interaction of antigen and cell bound antibody, is often considered to be accompanied by intravascular clotting leading to defibrination syndrome[1,2]. In experimental anaphylactic conditions, the activation and consumption of Hageman factor[3], factors XI and IX[4], the activation of fibrinolytic system[5], a moderate fall in fibrinogen concentration[4,5] and the appearance of fibrinogen degradation products[6] have been demonstrated. However, direct studies concerning the detection of fibrin formation have not been carried out. Our present results provide direct evidence that no intravascular fibrin formation occurs in cell bound IgE mediated anaphylactic shock of rats, while in the same species thrombin injection induced a marked and the addition of ellagic acid (an activator of Hageman factor) a transient intravascular clotting.

Materials and methods. Wistar male rats (150–200 g each) were sensitized with horse serum and *Pertussis* vaccine, as had been previously described[5,6], and were submitted to anaphylactic shock 12 days later by i.v. administration of 1 ml horse serum[5,6]. One group of the sensitized animals was treated with 100 mg/kg heparin i.v. 30 min before antigenic challenge. One control group was injected i.v. with bovine thrombin (Topostasin, Roche, 200 NIH units per 4 ml saline/kg) or ellagic acid (Koch-Light, 40 mg per 4 ml saline/kg). Then, 10 min before antigen, thrombin or ellagic acid injections, 2 ml/kg of a solution containing 2 mg/ml ^{125}I labelled[7] (5 μCi/mg) bovine fibrinogen (Armour Pharm., purified further by the method of LAKI[8]) and 0.6 mg per ml ^{131}I labelled[7] (20 μCi/mg) bovine serum albumin was injected into each animal through a tail vein. At various intervals following the addition of antigen, thrombin or ellagic acid, blood samples were obtained from the abdominal aorta, then one lung, one kideny, the spleen, a piece of liver and small intestine were quickly removed for the determination of their ^{125}I and ^{131}I radioactivity by a Gamma NK-350 counter. Plasma radioactivities, the amount of fibrin in organs, and the organ per blood ^{125}I and ^{131}I ratios were calculated as described by Busch et al.[9]. The ethanol gelation test was carried out by the method of Godal and Abildgaard[10].

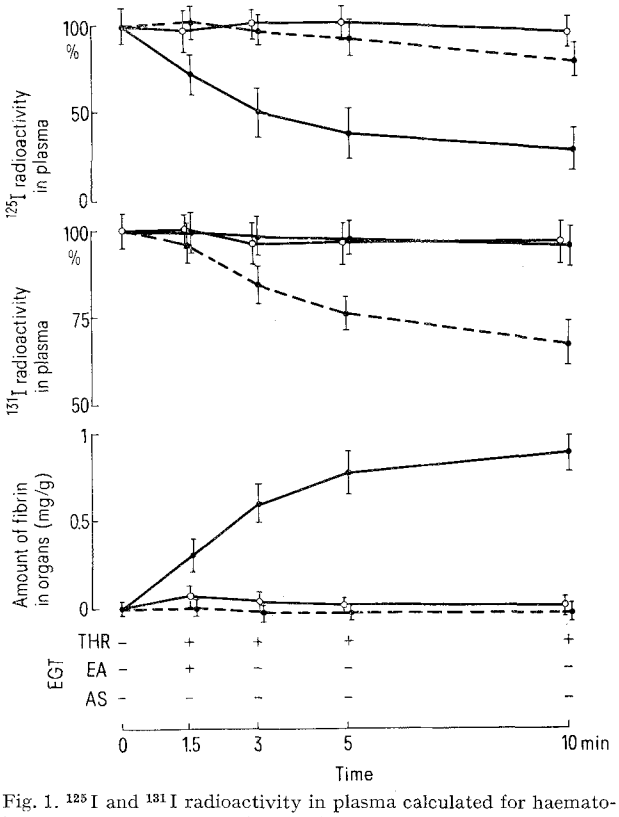

Fig. 1. ^{125}I and ^{131}I radioactivity in plasma calculated for haematokrit values, the amount of fibrin in organs and the results of the ethanol gelation test (EGT) at various intervals following the challenge of anaphylactic shock (AS, ●——●), the injection of thrombin (THR, ●—●), or ellagic acid (EA, ○—○). Each point represents 3 experiments (mean ± SE).

Results and discussion. A progressive and dramatic fall in the fibrinogen level, positive ethanol gelation test and high amount of fibrin in the organs (its distribution in the 10th min: lung 51,3%, small intestine 22,1%, liver 16,9%, kidney 7%, spleen 2,7%) could be observed following thrombin addition (Figure 1). In case of anaphylactic shock, the fibrinogen and the bovine serum albumin content of the plasma decreased slowly and in parallel, which is due to the diluting effect of the so-called 'excess plasma' operating as the shock proceeds[11]. The ethanol gelation test was negative and no sign of fibrin formation was found after anaphylactic challenge (Figure 1). However, a possibility of overlooking fibrin deposition in organs could be evoked, because the fibrin content of the organs was calculated by using the ^{131}I bovine serum albumin content of the organs as a plasma marker[9], although the rate of anaphylactic increase of permeability may be different for fibrinogen and bovine serum albumin. Therefore the organ per blood ratios of ^{125}I and ^{131}I radioactivity were determined during anaphylactic shock and found to change strictly in parallel (Figure 2) in the lung and the small intestine (the shock organs with haemorrhage[5]) as

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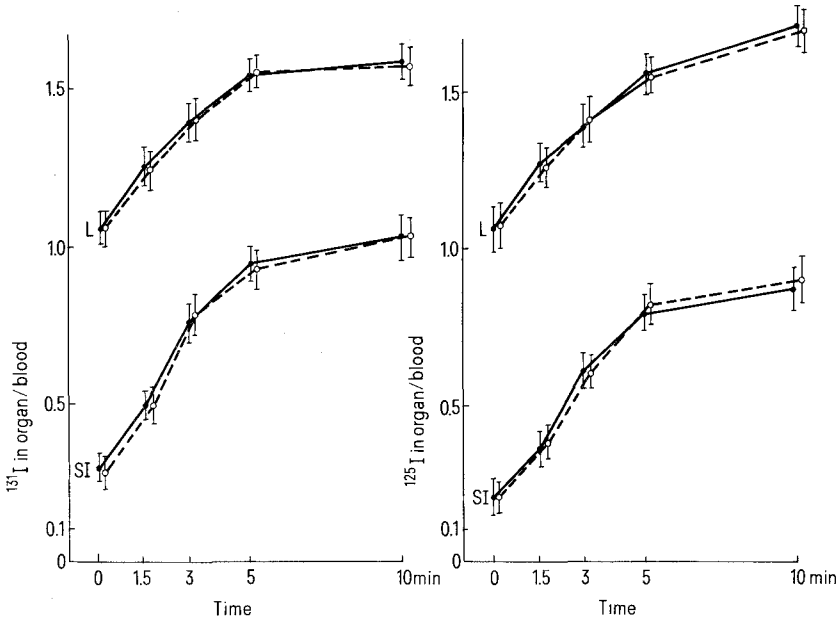

Fig. 2. Organ per blood ratios in the lung (L) and the small intestine (SI) of saline (●—●) or heparin (○—○) treated rats at various intervals after the challenge of anaphylactic shock. Each point represents the mean ± SE of 3 experiments.

well as in other organs (not included in Figure 2) of heparinized and non-heparinized rats. These findings mean that intravascular clotting as an etiological factor in the anaphylactic shock of the rat can be excluded.

A high dose of ellagic acid, a potent activator of Hageman factor, was necessary for even a transient fibrin formation (Figure 1); the distribution of the fibrin formed in the 1.5th min: liver 72%, lung 18%, small intestine 10%, which was readily eliminated later probably by the activated fibrinolytic system[3]. The anaphylactic results presented above show that the anaphylactic activation of Hageman factor, which we have demonstrated earlier in the rat[3], does not result in fibrin formation, although several additional etiological factors of hypercoagulability, such as anoxia and anoxaemia, endothelial damage, proteolytic enzymes and activated complement[12] are also existing. Consequently, highly potent anticoagulant activities are present or emerged even in the very early phase of anaphylactic shock, preventing intravascular clotting, and on the base of our present knowledge, it may be concluded that the slightly decreased fibrinogen level[4,5], the appearance of fibrinogen degradation products[6] and the marked hypocoagolability in the later phase of anaphylactic shock[3,4] are the consequences of the primary activation of fibrinolytic system[3,5].

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Comparison of a Natural Heparinoid with Sodium and Calcium Heparin for their Effect on the Inhibitor of Activated Factor X

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Summary. The reaction between activated factor X (Xa) and its natural inhibitor (XaI) was accelerated in vitro by both sodium heparin and an heparinoid, which was about 3 times less potent than heparin. The s. c. administration in humans of 5,000 units of sodium and calcium heparin was followed by the detection of a plasma activity potentiating XaI. In the majority of subjects, the heparinoid was not effective. These observations indicate that the use of heparinoids should not be considered as an alternative to heparin in the prevention of thromboembolism.

In the last few years heparinoids have received increasing attention for their potential applicability in the prevention and management of thrombosis and atherosclerosis. These compounds are acid mucopolysaccharides obtained by extraction from animal and vegetal sources or by semisynthetic procedures. Investigations largely carried out in animals have shown that they protect against experimental hyperlipidemia[1], release clearing factor into the blood stream[2], enhance fibrinolysis[3–5] and inhibit platelet aggregation[6–8], whereas blood coagulation screening tests[2,9] are hardly affected. However, small doses of heparin (0.2–0.01 U/ml) which are without effect on such coagulation tests, potentiate the natural inhibitor of activated factor X[10–12]. Since this is presently considered a key factor in the development of thrombosis[13], it would be of interest to know whether heparinoids mimick the effect of minidose heparin and enhance the plasma inhibitor activity. In this study, we have compared sodium and calcium heparin with a natural heparinoid extracted from pig duodenum, both in vitro and following their s.c. administration in human volunteers.

Materials and methods. The investigation was carried out in 6 healthy persons (aged from 24 to 33 years) who gave informed consent. The drugs tested were calcium