Lymphatic Fibrinolysis in Rats

Although the clotting capacity of lymph and the concentration of clotting factors are well investigated[1,2], only a few data are available concerning the fibrinolytic system in lymph of various mammals[3,4]. In this report the fibrinolytic components in lymph of rats were studied, beginning with the end of the cannulation procedure of the thoracic duct until 7 days after operation.

Materials and methods. Female rats of a Wistar strain, weighing from 200–250 g were used. According to a technique described by BOLLMAN, CAIN and GRINDLAY[5], the thoracic duct of these animals was cannulated in its abdominal part with a polyethylene cannula. During the time of observation, the animals were kept in cages specially designed similar to those described by BOLLMAN[6]. Lymph specimens were sampled within 1 h in cold glass tubes containing $^1/_{10}$ volume of a heparin solution. Citrated blood was obtained by aortic puncture. Estimations were carried out immediately after sampling.

Plasminogen was determined according to the caseinolytic method of ALKJAERSIG et al.[7], with application of the LOWRY-technique. 1 U/ml is defined as the tyrosin amount, emerged from a 2% casein solution, that gives an O.D. of 0.001 in our laboratory conditions. The antiplasmin level was determined in a caseinolytic assay too, 1 U/ml being 1% proteinolysis inhibition of a porcine plasmin standard.

Plasmin activity on heated fibrin-agar plates was determined according to the modified method of ASTRUP and MÜLLERTZ[8]. The protein concentration of each lymph specimen was determined according to the Biuret method.

Results. The results of the measurements of the fibrinolytic activity of lymph on heated fibrin-agar plates are shown in Figure 1. In 16 of 22 operated animals, we observed a distinct fibrinolytic activity within the first 8 h after operation. The lysed areas in these specimens ranged from 110 to 260 mm², the protein concentration being between 1.8 g/100 ml and 2.5 g/100 ml. On the first day after cannulation, only 1 of the 24 animals showed a fibrinolytic activity, the lysed area being 70 mm² and the protein concentration 1.1 g/100 ml. The number of animals showing fibrinolytic activity increased continuously from day 1 until day 7 after cannulation. At this time we observed lysed areas ranging from 110 to 320 mm² in 18 from 20 animals. The protein concentrations were between 0.7 and 1.9 g/100 ml.

The first blood specimen, obtained 4–6 h after operation, showed enzymatic activity in 8 out of 10 animals. The lysed areas of plasma samples ranged from 50 to 75 mm², the protein concentration being about 3.5 g/100 ml. No fibrinolytic activity was observed in the plasma during the following 7 days.

The Table summarizes the averages of the plasminogen and antiplasmin levels in lymph and plasma on various days. The levels are always lower in lymph than in plasma. The plasminogen level of normal rat plasma is about 227 U/ml (S.D. ±26 U/ml), the antiplasmin content about 70 U/ml (S.D. ±9 U/ml). A comparison of plasminogen and antiplasmin levels in lymph is difficult because of the varying lymph flow rates and protein concentrations. As plasminogen and antiplasmin are proteins too, we expected a correlation between the protein concentration of lymph and the plasminogen and antiplasmin activity. The partial correlation of protein concentration to plasminogen activity was highly significant ($r = 0.355$; $p = 0.005$). The partial correlation of protein concentration to antiplasmin was even higher ($r = 0.536$; $p = 0.0005$). In respect to these correlations, it seems reasonable to plot the plasminogen and antiplasmin levels of lymph and plasma against the protein content of lymph and plasma and to give the data expressed as 'Units/g protein'.

The plasminogen levels, expressed as Units/g protein, are higher in lymph than in plasma (Figure 2). The levels increase both in lymph and plasma, but in lymph the increase is much stronger. On day 1 after operation, when the fibrinolytic activity is low, there is a decrease of plasminogen in lymph and plasma.

This may indicate a consumption of plasminogen, following the operation-induced activation of the fibrinolysis. The antiplasmin levels (Units/g protein) are lower

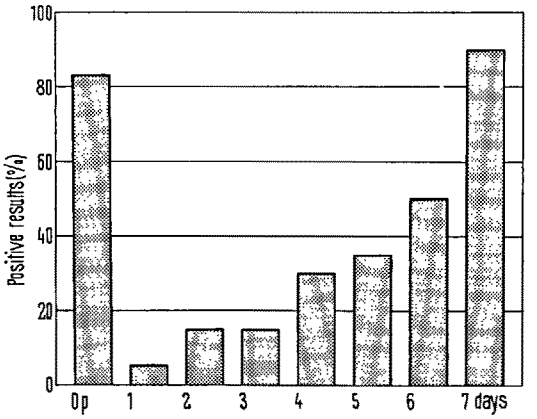

Fig. 1. Positive results of lymph samples on heated fibrin-agar plates during 7 days of thoracic duct cannulation.

Averages of plasminogen and antiplasmin levels (Units per ml) in lymph and plasma

	3–6 h after cannulation $n = 10$		Day 1 after cannulation $n = 10$		Day 7 after cannulation $n = 8$	
	Plasminogen	Antiplasmin	Plasminogen	Antiplasmin	Plasminogen	Antiplasmin
Lymph	105	23	60	14	74	27
Plasma	199	57	127	56	252	74

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in the lymph than in the plasma from 4 h until one day after operation (Figure 3). Later on, the antiplasmin levels in lymph and plasma do not differ significantly.

The results of our investigations reveal a high fibrinolytic activity in thoracic duct lymph of rats. The variations of this activity in the course of the cannulation allow 2 kinds of interpretation: 1. The first peak in activity is due to the stress of operation. It is well known[9–11] that stress enhances the activation of fibrinolysis in various mammals. After a phase of depletion, there is a second stress-induced peak caused by the confinement in the narrow cages and the manipulations during the sampling periods. 2. Following a stress-induced activation of the fibrinolytic system in lymph and plasma, there is a phase of depletion. On day 6 and 7 after operation the fibrinolytic activity returns to normal values. Our results in these days therefore indicate an activation of the lymphatic fibrinolytic system even in the preoperative state.

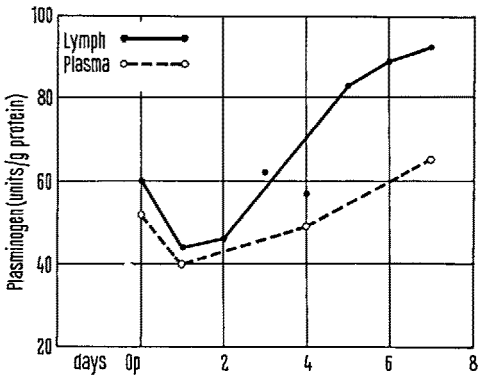

Fig. 2. Plasminogen levels in lymph and plasma of rats during 7 days of thoracic duct cannulation.

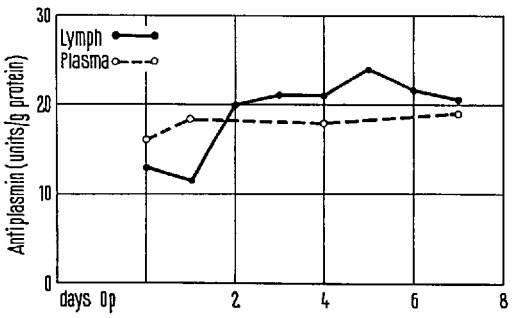

Fig. 3. Antiplasmin levels in lymph and plasma of rats during 7 days of thoracic duct cannulation.

Whatever kind of interpretation may be applied, the results reveal that, at least at some times, the fibrinolytic activity in rat lymph exceeds that of plasma. These findings are in part explained by the higher plasminogen-antiplasmin ratio in lymph, compared with that of plasma. A high plasminogen-antiplasmin ratio in lymph will enhance the fibrinolysis, but plasmin will be present only if an activation of plasminogen has taken place. BEARD et al.[10] stated that after stress the lysosomes in the rat liver release plasminogen activators into the body fluids. One could suggest that the activator reaches the blood stream not directly, but by the way of the lymphatic system. Following this hypothesis, the lymphatic system of rats would play a part in the activation of plasmatic fibrinolysis after stress.

The statement of a high fibrinolytic activity in lymph is in contrast to other investigations performed on dogs[3] and humans[1]. In these reports, however, no data are given as to what time has elapsed between cannulation and lymph sampling. It is possible that the lymph has been sampled in the phase of depletion where the fibrinolytic activity is low. The fibrinolytic system of rats, on the other hand, may behave differently to that of dogs and humans[12].

Zusammenfassung. Ratten zeigen regelmässig zu bestimmten Zeiten nach Ductus-Thoracicus-Kannulierung eine hohe, unter Umständen durch Stress induzierte fibrinolytische Aktivität in der Lymphe; diese Aktivität braucht nicht von einer plasmatischen Fibrinolyse begleitet zu sein.

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Immunological Reactivity of a Synthetic Polymannan

In recent years, the water-soluble polysaccharides (galactomannans, mannan, and glucans) of a number of dermatophytes have been studied in this laboratory[1]. Each species investigated contains a galactomannan I, galactomannan II and a glucan, except *Trichophyton rubrum*, which yielded a mannan instead of a galactomannan I. The molecular weights of these polysaccharides are about 10,000.

A synthetic polymannan, free of nitrogen, was obtained from Dr. C. SCHUERCH. It consists of α1→6 linked D-mannopyranose units resembling the basic chain of the dermatophyte galactomannans I, but has a molecular weight of 40,000[2]. It was, therefore, of interest to study the ability of this synthetic polymannan to induce cutaneous hypersensitivity and humoral antibody formation.

The polymannan was dissolved in sterile saline and emulsified in an equal volume of Freund's complete adjuvant for immunization of guinea-pigs and mice.

Hartley strain guinea-pigs were injected with 2.0 mg (Group I) or 4.0 mg (Group II) of synthetic polymannan in Freund's complete adjuvant. A total volume of 1.0 cm^3, 0.5 cm^3 s.c. in the nape of the neck and 0.5 cm^3 in the hind foot pads was injected. 21 days later, guinea-