EFFECT OF A PREPARATION OF ASPERGILLIN-O TYPE FROM Aspergillus oryzae ON CAPILLARY PERMEABILITY IN RABBITS

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Many attempts have been made to study the mechanism of capillary permeability [1, 3-6] but no final solution to the problem has been obtained. According to an interesting hypothesis [6], the internal surface of the blood vessels is covered with a thin fibrin film. The increase in capillary permeability during the action of some substances (bradykinin and plasminogen) is attributed by the author of this hypothesis [5] to activation of the fibrinolytic system, destroying the integrity of the fibrin film. This view could be confirmed objectively by an experiment in which a preparation having a direct fibrinolytic action, independent of the fibrinolytic system of the body, was injected into the blood stream.

In the present investigation the action of a preparation possessing fibrinolytic properties on capillary permeability was studied.

EXPERIMENTAL METHOD

The agent having a direct fibrinolytic action used in the experiments was a preparation of aspergillin-O type. Aspergillin-O was first obtained in 1958 [8], and subsequent investigations determined its properties [9, 10]. A preparation of the same type was obtained in 1963 by Kudryashov and Andreenko and co-workers [2]. They showed that the preparation possesses not only fibrinolytic, but also fibrinogenolytic activity.

The author obtained a preparation of aspergillin-O type by the method of Stefanini and co-workers [8, 9] from the culture fluid of the mold Aspergillus oryzae (strain obtained from the Institute of Microbiology, Academy of Medical Sciences USSR). The preparation is a white amorphous fluffy substance, hygroscopic, and giving positive reactions for peptide bonds and α-amino acids. LD_{50} for the preparation from A. oryzae by intravenous injection into rabbits was 16.8 mg/kg. In an experiment in vitro the preparation dissolved plasma clots in a dilution of 1 : 8 from an initial concentration of 10 mg/ml.

Experiments were carried out on chinchilla rabbits weighing 4 kg. The capillary permeability in the rabbits was measured from the change in the Congo red index in the serum of the experimental animal after injection of the preparation from A. oryzae. The control consisted of the same rabbits, receiving injections of the dye only in preliminary experiments. The fibrinolytic activity of the blood was determined by the method of lysis of the plasma euglobulins [7].

The experiments were carried out as follows. A 1% aqueous solution of Congo red was injected into a vein of the rabbit's ear in a dose of 1 ml/kg body weight. In control experiments blood samples for obtaining serum were taken from the marginal vein of the other ear 4, 30, 60, and 120 min after injection of the Congo red solution. The first sample, regarded as the original sample, was taken from the experimental rabbits 4 min after injection of the dye, and 30 min after the injection, the preparation from A. oryzae was injected intravenously in a dose of 12 mg/kg body weight. A blood sample was then taken quickly from the marginal vein of the other ear. Subsequent blood samples were taken 60 and 120 min after injection of the dye, i.e., 30 and 90 min after injection of the preparation.

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Table 1. Fibrinolytic Activity of Plasma of Rabbits After Injection of Preparation from A. oryzae

Rabbit No.	Time of taking samples of plasma after injection of preparation (min)	Time of lysis of clots (min)	
		expt.	control
1465	5 30 90	25—45	360
961	5 30 90 150	30—45	300
824	30 90	40—45	360

Table 2. Congo Red Index of Rabbits Receiving Preparation from A. oryzae (results of experiments on 5 animals; M ± m)

Time of taking blood samples from moment of injection of dye (min)	Congo red index		P
	control (dye only injected)	Expt. (dye + preparation inject.)	
4	100	100	
30	85±4,5	82±5,2	>0,05
60	66,7±4,8	67±7,4	>0,05
120	36,5±2,0	31,8±4,7	>0,05

The concentration of Congo red in the serum was determined from the optical density on the FEK-M photoelectric colorimeter using a green filter, in a cuvette 5 mm wide. The serum was first diluted in the ratio 1:3 with physiological saline. The standard for comparison was serum without dye in the same dilution.

EXPERIMENTAL RESULTS

Results for the fibrinolytic activity of the plasma of the rabbits receiving the preparation from the mold A. oryzae intravenously are given in Table 1. It is clear from these results that the preparation accelerates fibrinolysis.

Results showing the changes in the Congo red index of rabbits receiving the preparation from A. oryzae are given in Table 2.

It follows from Table 2 that a comparison of the control and the experimental rabbits receiving the preparation from A. oryzae showed no significant difference between the rate of disappearance of the dye from the blood serum.

The results obtained do not confirm the theoretical views [5] that the internal surface of the blood vessels is covered with a thin fibrin film, for the preparation now tested, a proteinase of fungal origin, had a direct fibrinolytic action on fibrin clots in experiments in vitro and stimulated the fibrinolytic activity of the blood in experiments in vivo; yet nevertheless it did not increase vascular permeability.

A similar conclusion was reached by other authors [4] who, in analogous experiments on dogs, used heparin and nicotinic acid as agents increasing the fibrinolytic activity of the blood. It follows from the results of these experiments that the vascular permeability was not disturbed in these circumstances. The authors cited remark that their investigations cast doubt on the existence of a fibrin film on the internal surface of the blood vessels, and also on the participation of fibrin in the mechanism of normal vascular permeability.

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