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EFFECT OF HEPARIN ON LIPID PEROXIDATION REACTION OF ERYTHROCYTES AND THEIR RESISTANCE

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In a physiological concentration (10 units/ml) heparin activates the ascorbate-dependent lipid peroxidation of lipids and reduces their resistance in citrate–phosphate buffer, pH 3.0. In a concentration of 100 units/ml heparin does not affect thermal (62°C) oxidation of methyloleate; this is evidence that heparin is not a direct-action prooxidant.

KEY WORDS: heparin; erythrocytes; peroxidation of lipids.

Heparin has been shown to participate in the humoral regulation of metabolism as a universal inhibitor of many enzymic processes [6, 12, 17]. The biological effect of heparin is due to its polyanionic structure and its marked ability to form complexes. When adsorbed on cell membranes heparin essentially changes their physicochemical properties, and thereby regulates the permeability of the membranes, their charge, and the cell metabolism [13, 14, 16]. According to some workers, heparin increases the osmotic and reduces the acid resistance of erythrocytes [7]; other workers conclude that it destabilizes cell membranes, evidently by activating phospholipase A_1 [20]. This enzyme removes the free fatty acids from structural phospholipids, and in conjunction with its actions on permeability and the charge, this may lead to activation of lipid peroxidation processes (LPP). LPP are known to play an important role in the regulation of the physicochemical properties of membranes under normal and pathological conditions [3, 4, 8]. A stationary level of LPP in the body is essential for its normal function and it is controlled by a whole series of factors [5], including hormones [19]. According to the present writers, LPP can be controlled by heparin, which activates the lipolipid system [18], on the one hand, and blocks glutathione reductase activity on the other hand [1].

The object of the present investigation was to study the possible effect of heparin on LPP of erythrocytes and their stability and also the anti- and prooxidant properties of heparin.

EXPERIMENTAL METHOD

Blood was taken from fasting donors into sodium oxalate and washed twice with physiological saline. Heparin (Richter) was first purified with ether. The erythrocytes were incubated for 10 min at 37°C with 10

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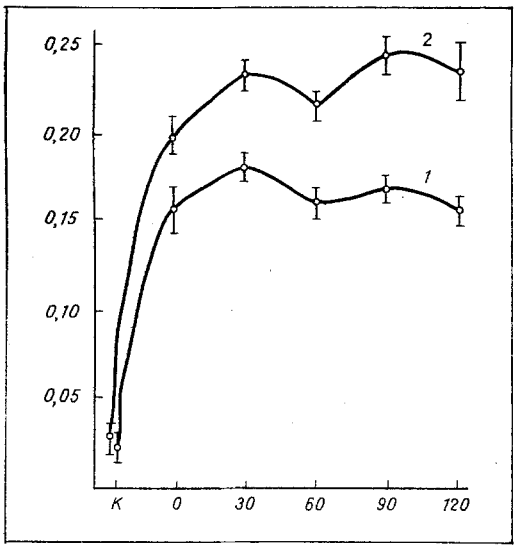

Fig. 1

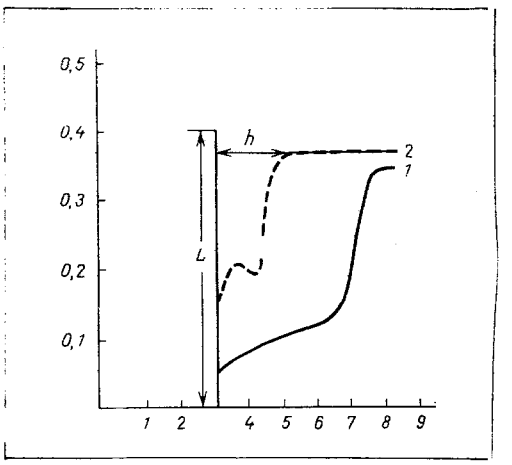

Fig. 2

Fig. 1. Effect of heparin (10 units/ml) on velocity of lipid peroxidation of erythrocytes: 1) control; 2) experiments. Abscissa, incubation time (in min). Ordinate, extinction at 233 nm.

Fig. 2. Effect of heparin (10 units/ml) on resistance of donors' erythrocytes in citrate–phosphate buffer, pH 3.0: h) time of hemolysis; L) light transmission of erythrocyte suspension in course of hemolysis. Remainder of legend as in Fig. 1.

TABLE 1. Effect of Heparin (100 units/ml) on Thermal (62°C) Oxidation of Methyoleate (M ± m)

Oxidation time, h	Control	Experiments	P
0	7 0	7,0	
2	12,2±0,26	12,8±0,12	>0,1
4	17,0±0,024	17,0±0,1	>0,1
6	21,8±0,6	22,9±0,5	>0,1

units/ml heparin in phosphate buffer, pH 7.4, and after a control sampling, 0.8 mM ascorbate, $12 \cdot 10^{-6}$ M $Fe(NH_4)_2SO_4 \cdot 5H_2O$, and 5 mg/ml of an alcoholic solution of vitamin D_2 were added to the incubation mixture [15]. Samples were then taken every 30 min for 2 h. The level of hydroperoxides [9] was used as an indicator of the velocity of the LPP of the erythrocytes. The effect of heparin on resistance of the erythrocytes in citrate–phosphate buffer, pH 3.0, was sutided by the original method [10]. To study whether heparin has any direct anti- or prooxidant action on LPP, its effect on the velocity of thermal oxidation of methyloleate was studied on a model suggested by Burlakova et al. [2]. Thermal (62°C) oxidation of methyloleate was carried out in the presence of 100 units/ml heparin. The rate of accumulation of hydroperoxides was determined by amperometric titration [11].

EXPERIMENTAL RESULTS AND DISCUSSION

The results of 10 experiments showed that heparin increases ($P < 0.05$) the velocity of ascorbate-dependent lipid perooxidation of erythrocytes (Fig. 1).

Activation of LPP by heparin could be one cause of destablization of the erythrocytes membrane and for that reason the resistance of erythrocytes was investigated in citrate–phosphate buffer, pH 3.0. The results showed that after incubation for 10 min with heparin the resistance of the erythrocytes fell significantly ($P < 0.001$; Fig. 2).

Meanwhile heparin, in a concentration of 100 units/ml, did not affect the rate of thermal oxidation of methyloleate. No significant difference was found between the experiment and control for a period of 6 h (Table 1). This indicates that heparin does not possess the properties of a direct anti- or prooxidant.

In physiological concentrations heparin thus activates LPP of erythrocytes in vitro; its action is evidently mediated through systems controlling the activity of LPP in cell membranes.

A further study of the prooxidant properties of heparin will help to shed light on the mechanism of its effect on the permeability of cell membranes and will spell out the indications for its use in states accompanied by a hyperperoxidation syndrome.

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