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# CORRELATION BETWEEN BLOOD FREE FATTY ACID CONCENTRATION AND PLATELET ACCUMULATION IN THE MYOCARDIAL CIRCULATION AFTER INJECTION OF ADRENALIN

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During prolonged emotional stress disturbance of the microcirculation may play an important role in damage to the heart [5]. The writer showed previously that besides activation of the sympathico-adrenal system and an increase in the blood level of free fatty acids (FFA) the number of platelets in the microcirculation of the myocardium is increased in animals with emotional-pain stress [1]. In stress the blood adrenalin concentration is increased by more than 8 times [9].

This paper gives data on disturbance of microcirculatory-platelet hemostasis in the heart muscle following injection of adrenalin into intact animals and when its lipolytic effect is modified with the aid of nicotinic acid and heparin. Nicotinic acid is known to depress basal and catecholamine-induced lipolysis and to cause a decrease in the blood FFA concentration [8]. Heparin liberates enzymes hydrolyzing lipids from the tissues into the blood stream and, as a result, the blood FFA level rises considerably [11].

## EXPERIMENTAL METHODS

Experiments were carried out on 25 rabbits. To isolate platelets blood was taken from the jugular vein. After isolation of the platelets by the usual method [6] plasma was added to the platelet residue and it was incubated with sodium $^{51}$Cr-chromate (specific activity $9.2 \cdot 10^5$ Bq/ml). Labeled platelets were suspended in plasma and injected into animals. The animals then received an intravenous injection of adrenalin in a dose of 1.2 μg/kg/min over a period of 30 min. Heparin (500 i.u./kg) and nicotinic acid (5 mg/kg) were injected intravenously 10 min before adrenalin infusion. To determine the blood content in the myocardium, $^{99m}$Tc-albumin was injected into the animals immediately after the end of adrenalin infusion. The animals were killed 5 min after injection of the nuclide. The blood content ($V_T$) in the heart muscle was calculated by the equation $V_T = (V_B \cdot C_T)/C_B$, where $V_T$ stands for the total blood volume; $C_B$ and $C_T$ the radioactivity of blood and tissue respectively (relatively to $^{99m}$Tc). The number of platelets in the blood was counted by a phase-contest method and the radioactivity of samples of blood and heart muscle tissue was measured, after which the numner of platelets in the myocardial circulation ($P_T$) was calculated by the equation: $P_T = (^{51}Cr_T/^{51}Cr_B) \cdot P_B$, where $^{51}Cr_T$ is the radioactivity of the myocardial tissue, $^{51}Cr_B$ the radioactivity of the blood; $P_B$ the number of platelets in the blood. Accumulation of platelets ($A_P$) in the myocardial circulation was judged from the difference between the number of platelets actually found ($P_T$) and the number which ought to have been found in the myocardial circulation to correspond to the quantity of blood contained in it ($V_T$): $A_T = P_T - V_T \cdot P_B$, where $P_T$ is the number of platelets in the myocardial circulation, $V_T$ the blood volume in the

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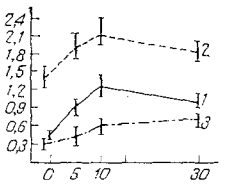


Fig. 1. Effect of adrenalin on blood FFA concentration after preliminary changes in the intensity of lipolysis. Abscissa, time (in min); ordinate, FFA concentration (in mM). 1) Adrenalin; 2) heparin + adrenalin; 3) nicotinic acid + adrenalin.

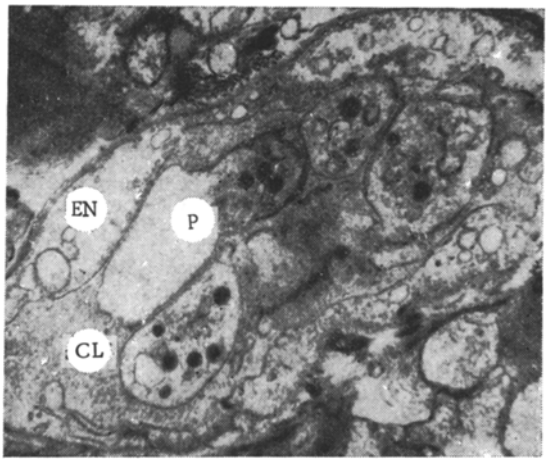


Fig. 2. Accumulation of platelets in capillary lumen after injection of adrenalin preceded by heparin. CL) Capillary lumen; EN) endotheliocyte; P) platelet, 7000 ×.

TABLE 1. Accumulation of Platelets in Myocardial Blood Vessels after Injection of Adrenalin into Intact Animals and Changes in Intensity of Lipolysis (M ± m)

| Parameter | Control | Adrenalin | Adrenalin + heparin | Adrenalin + nicotinic acid |
|---|---|---|---|---|
| Number of platelets in blood, $\cdot 10^6$/ml | 269±8 | 183±11 | 178±12 | 241±9 |
| *P* | | <0,001 | <0,001 | <0,05 |
| Number of platelets in myocardial blood vessels, $\cdot 10^5$/g tissue | 221±9 | 669±48 | 1502±63 | 347±28 |
| *P* | | <0,001 | <0,001 | <0,01 |
| Blood volume in myocardium, μl/g tissue | 81,2±1,7 | 73,3±1,6 | 70,3±1,9 | 83,4±1,2 |
| *P* | | <0,02 | <0,01 | |
| Accumulation of platelets in myocardial blood vessels, $\cdot 10^5$/g tissue | 0 | 536±51 | 1377±62 | 143±32 |

myocardium, and $P_B$ the number of platelets in the blood. The FFA concentration in the blood was determined by a colorimetric method [7].

Pieces of myocardium from the left ventricle were taken for electron-microscopic investigation and fixed in 1% osmium tetroxide solution. Sections were studied in the UEMB-100B electron microscope.

## EXPERIMENTAL RESULTS

Graphs of the lipolytic action of the adrenalin in intact animals and after administration of heparin or nicotinic acid are given in Fig. 1. After injection of adrenalin there

was a marked increase in the blood FFA concentration. Meanwhile the number of platelets in the blood decreased (Table 1). The coefficient of correlation between the changes in the platelet count and blood FFA level was 0.57 ($P < 0.05$). Accumulation of platelets was observed in the myocardial blood vessels under these circumstances (Table 1).

In full agreement with data in the literature [4] injection of heparin was followed by a sharp increase in the blood FFA concentration. Against this background adrenalin caused an even greater increase in the blood FFA concentration. In the animals of this group the largest decrease was observed in the blood platelet count. Accumulation of platelets in the myocardial blood vessels averaged $(1377 \pm 62) \cdot 10^5$/g tissue. Platelets in a state of adhesion and aggregation were found electron-microscopically in the capillary lumen (Fig. 2). These observations indicate that the accumulation of platelets discovered by the radionuclide method was mainly connected with their aggregation in the lumen of the myocardial microcirculation.

In the experiments in which adrenalin was injected and the intensity of lipolysis and the rate of mobilization of FFA from the fat depots were depressed by nicotinic acid, accumulation of platelets in the myocardial blood vessels was least marked.

The results thus indicate close correlation between the increase in FFA concentration, the decrease in the blood platelet count, and accumulation of platelets in the myocardial blood vessels. It can be concluded from the results of the experiments with a change in the lipolytic action of adrenalin as a result of administration of nicotinic acid and heparin that one of the pathogenetic factors involved in platelet accumulation in the microcirculation of the myocardium is an increase in the blood FFA concentration.

Recent experiments *in vitro* have shown that saturated and unsaturated FFA increase the aggregating power of the platelets considerably [10]. It can be tentatively suggested that in the present experiments the ability of the platelets to undergo aggregation and adhesion was considerably increased by the FFA, and the most active fraction of the platelets in this respect aggregated in the capillary lumen. It must also be recalled that because of the cardiotoxic action of catecholamines, they increase the catabolism of membrane phospholipids in the heart muscle so that the concentration of lysophospholipids is increased [1]. These substances, like FFA, considerably increase platelet aggregation [2].

Finally, a contribution toward the increase in aggregating power of the platelets may probably have been made by the changes in the pressure gradient arising in the coronary vessels and in the chambers of the heart after injection of adrenalin. This suggestion is confirmed by data in the literature on a considerable increase in the sensitivity of platelets to aggregating stimuli under the influence of variable pressure [3].

It can be concluded that an increase in the blood FFA concentration and accumulation of platelets in the myocardial blood vessels play an important role in the pathogenesis of catecholamine-induced injuries of the heart.

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