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Platelet Adhesiveness in Cyanotic Congenital Heart Disease

By

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In cyanotic children with congenital heart disease an increased adhesiveness of platelets was observed. A statistically significant connection was demonstrated between platelet adhesiveness and hypoxaemia and the degree of polyglobulia. Some cyanotic children were hyperlactacidaemic. With the increase of the lactic acid concentration, the adhesiveness of platelets increases; this connection is not significant statistically.

When studying cellular haemostasis in children with congenital heart disease, we have observed that in conditions associated with cyanosis, susceptibility to agglutination and adhesion of platelets in vivo (wounding) and in vitro (glass surface) is sometimes increased [7].

Owing to the anatomic and haemodynamic conditions in patients with cyanotic heart disease (right to left shunt, mixing of venous and arterial blood, narrowing of pulmonary orifice, decreased pulmonary blood flow, etc.) the oxygen saturation of arterial blood is lower than normal. Chronic hypoxaemia induces a chain of compensatory processes. Secondary polyglobulia develops and thus the viscosity of blood increases and its circulation is slowed down. Tissular metabolism is shifted in anaerobic direction, lactic acid accumulates and metabolic acidosis develops [8]. These changes may influence the adhesiveness of platelets [9, 10, 12].

In the following we shall report on examinations performed in order to elucidate the role of hypoxaemia, polyglobulia and hyperlactacidaemia in the development of increased platelet adhesiveness.

Materials and Methods

The investigations were made on 36 cyanotic children suffering from various cardiac malformations (28 cases of tetralogy of Fallot, 2 cases of pentalogy of Fallot, and 6 cases of transposition of the great vessels). The patients were of both sexes, aged between 3 and 14 years. Arterial oxygen saturation ranged between 40 and 90%, the packed cell volume from 48 to 90%. During and for several weeks before the examinations the children were not given digitalis and drugs influencing coagulation or platelet function.

The examinations were carried out in the late morning hours. Samples of venous blood were collected from the cubital vein, of arterial blood from the femoral vein or the aorta during cardiac catheterization. For the study of platelet adhesiveness, blood was collected in a plastic tube con-

taining 3.8% trisodium citrate. The quantity of anticoagulant was corrected according to the haematocrit value, in order to stabilize the rate of plasma : citrate [5].

Platelet rich plasma (PRP) was obtained by centrifugation of citrated blood at 800 r.p.m. for 5 min, *platelet poor plasma* (PPP) by centrifugation of PRP at 3500 r.p.m. for 15 min. The platelet count was adjusted with PPP to 400,000 cu.mm.

Haematocrit was determined in microhaematocrit tubes centrifuged at 10,000 g for 6 min using sodium oxalate as anticoagulant.

Platelet adhesiveness to glass was measured according to the method of HELLEM [9]. Citrated blood was left standing at room temperature for 40—50 min and then passed at constant speed through a standardized glass bead column. Contact time between blood and glass beads was 30 sec. Platelets were counted before and after exposure to glass and the percentage of platelets retained in the column was calculated. The mean of two determinations was used.

Platelets were counted according to the method of FEISSLY and LÜDIN [3].

Arterial oxygen saturation (S_aO_2) was measured with an Atlas oxymeter.

Lactic acid concentration of blood was determined enzymatically with the use of UV. test (Sigma) [14].

With the exception of glass beads for platelet adhesiveness, silicone glass instruments were used (Silicone Lubricant, Dow-Corning Corp., USA).

Evaluation of results was carried out by means of Student's t test and the correlation coefficient. A p value of less than 0.05 was considered significant.

RESULTS

1. Oxygen saturation of arterial blood in the patients was between 40 and 90%. Thirty-three to ninety per cent of the platelets adhered to the glass surface. A negative correlation was observed between the two parameters (Fig. 1). In patients with low S_aO_2, the stickiness of platelets was increased. The correlation coefficient was $r = 0.93$ showing a mathematically significant connection between the two variables ($p < 0.01$).

2. The packed cell volume of patient blood was between 48 and 82%. Plotting the platelet adhesiveness in the function of the haematocrit value, a direct connection may be observed (Fig. 2A). The stickiness of platelets showed a considerable dispersion in the individual haematocrit fields. The correlation coefficient was $r = 0.37$ The connection between the valueswas significant mathematically ($p < 0.05$).

For the verification of the connection between haematocrit and platelet adhesiveness, experiments in vitro were carried out (Fig. 2B). Ten blood samples with raised packed cell volume were repeatedly diluted with autologous PRP. After the determination of thrombocyte adhesion we found that the decrease in packed cell volume involved a percentual fall of platelets retained on the glass beads. The change was significant ($p < 0.01$). On the basis of in vivo and in vitro data we calculated the regression equations, the comparison of which revealed a considerable similarity. The deviation between the two regression coefficients was not significant statistically, i.e. in the relation studied the two samples (in vivo and in vitro) had identical properties.

3. The blood lactic acid concentration in the cyanotic patients was

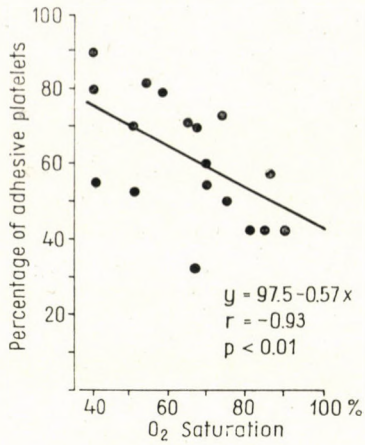

FIG. 1. Relationship between platelet adhesion in vitro and arterial oxygen saturation in cyanotic heart disease

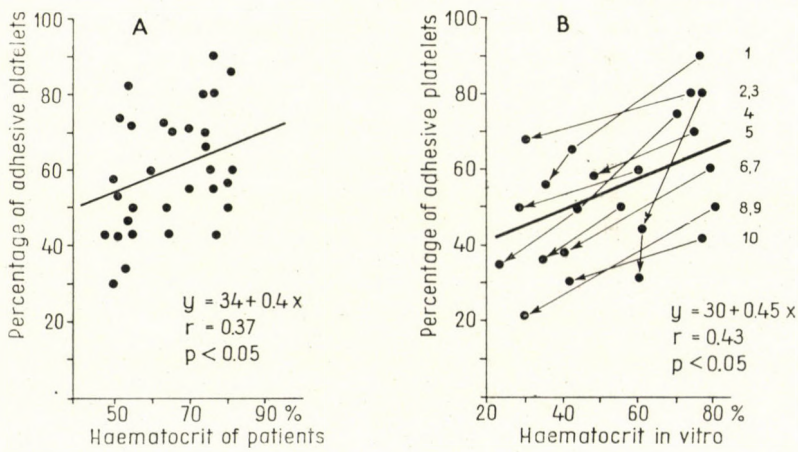

FIG. 2. (A) Relationship between haematocrit value and platelet adhesion in vitro in cyanotic congenital heart disease. (B) Effect of haematocrit on platelet adhesiveness in vitro. Platelet adhesiveness was measured in mixtures containing a constant quantity of PRP but varying proportions of PPP and packed red blood cells

higher than normal, between 12 and 71 mg/100 ml. The degree of hyperlactacidaemia influenced the number of platelets adhering to the glass bead column (Fig. 3). A linear correlation was observed between the two parameters, the connection was not significant ($p > 0.05$).

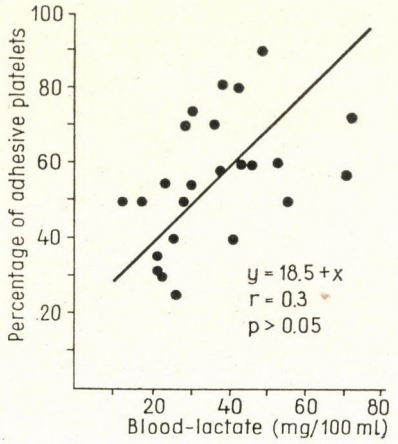

FIG. 3. Relationship between platelet adhesion in vitro and blood lactate level in cyanotic heart disease

DISCUSSION

In patients with cyanotic congenital heart disease, a comparatively asymptomatic, low grade, chronic disseminated intravascular coagulation may be found [6, 11]. This may explain the susceptibility to thrombosis and haemorrhage of these patients.

The platelets are primarily involved in the development of thrombosis. In adults it was demonstrated that the adhesiveness and aggregation of platelets is increased in the case of thromboembolic signs [1, 10]. In cyanotic cardiopathic children similar signs may be observed [7]. This suggested that the increased stickiness of platelets represents the pathophysiologic basis of the chronic DIC in these patients.

In children with cyanotic congenital heart disease, the most significant and fundamental pathologic sign is hypoxaemia, developing as a consequence of anatomic and haemodynamic conditions; the function of platelets may directly and also indirectly (consecutively) be influenced by the low oxygen tension. The intact metabolism of thrombocytes — especially glycolysis as the main source of energy (ATP-synthesis) — is indispensable for the normal function of platelets. With the inhibition of glycolysis and oxidative phosphorylation, severe disturbances of thrombocyte functions may be induced (retraction, aggregation, adhesion) [13]. Due to the lack of oxygen, we may reckon in our patients with a direct metabolic damage of platelets.

As a result of hypoxaemia, compensatory polyglobulia develops. The investigations of HELLEM [9] showed that the change of packed cell volume influences the adhesion of platelets on glass surface. He explained this by the fact that platelet adhesiveness is stimulated by a substance which he called factor R. This substance proved to be adenosine diphosphate (ADP)

released from the red blood cells [4]. In the case of polyglobulia, more erythrocytes are destroyed than normally, thus more ADP is released [10]. The phenomenon may be verified also in vitro. The stickiness of platelets showing increased adhesiveness in polyglobulic blood, is decreased after the blood is diluted with plasma.

The other consequence of hypoxaemia is the deviation of cellular metabolism in anaerobic direction. The end-product of anaerobic glycolysis is lactic acid, which accumulates in the cells and blood plasma. In chronic cyanosis, the consequence of hyperlactacidaemia is metabolic acidosis [8]. According to the observations of LABORIT and ORNELLAS [12], lactic acid increases significantly the adhesiveness of platelets, even at physiologic concentration (20 to 80 mg/100 ml).

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