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EFFECT OF BED REST ON THE ADENINE NUCLEOTIDES CONCENTRATION IN HUMAN BLOOD PLATELETS

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The effect of immobilization in bed on metabolism and function of human blood platelet was studied. Blood platelets taken from patients with bone fractures after long term bed rest (14 days and 28 days) demonstrated significantly reduced concentration of total adenine nucleotides (after 28 days reduction about 30%). This decrease of total platelet adenine nucleotides after immobilization in bed is probably caused by stimulation of platelet secretory process. Thrombin which released from control platelets 58.2%±1.5% of total adenine nucleotides liberated decreased amounts (only 23.1%±3.3% of total) of nucleotides from patient platelets isolated after 28 days of immobilization in bed. Loss of nucleotides from platelets was accompanied by slighly increased extent of platelet aggregation. It is concluded that during bed rest the reactivity of blood platelets (aggregation and release reaction) is stimulated.

Key words: *blood platelets, bed rest, aggregation, adenine nucleotides*

INTRODUCTION

Blood platelets are the smallest morphological elements of blood. Following any damage to blood vessels, platelets collect at the site of injury and undergo shape change with the aggregation and the release of certain substances from their granules (1, 2). Upon stimulation from the platelets adenine nucleotides are released (1—4). Adenine nucleotides in human blood platelets exist in two pools: a metabolic and a storage one (2, 3). The storaged nonmetabolic pool of platelet adenine nucleotides (about 60% of total platelt nucleotides, mainly ADP and ATP) is extruded from dense granules during activation of platelets induced by thrombin.

It is still unclear how bed rest may affect blood clotting system and act

on the blood platelet metabolism and function. Our preliminary study showed the effect of bed rest and physical exercise on antioxidative enzyme system in blood platelets (5—7). The purpose of the present study was to elucidate whether bed rest has any effect on the concentration of adenine nucleotides in human blood platelets and on ADP-induced aggregation of these cells.

MATERIALS AND METHODS

Healthy males (35—45 years) without systemic disease, hospitalized due to extremitaties fractures were studied. None of them was administrated any drugs before and during the performed study. Blood was taken from patients after 14 days and 28 days of bed rest. Blood (20 ml) was drown from antecubital vein into ACD solution (5 : 1, v/v). Blood platelets were isolated by differential centrifugation technique (8, 9). The control group consisted of 41 healthy individuals. The platelets were washed and suspended in buffered saline, pH 7.4, counted (Burker chamber) and treated immediately with 1.2 mol/l icecold perchloric acid (1 : 1, v/v), mixed and left for 2 hours at 4°C. After centrifugation in supernatants the total concentration of platelet adenine nucleotides was estimated spectrophotometrically (9). To determine the concentration of individual adenine nucleotides: ATP, ADP and AMP, the enzymatical test-combination (UV-method) according to Boehringer Mannheim Diagnostica was applied (10).

All surfaces being in contact with blood or cell suspension were siliconised or from plastic.

Thrombin-induced platelet release reaction: Samples of platelet suspension were incubated with thrombin (10 u/10^{10} platelets) for 5 min, at 37°C. After centrifugation, to the cooled supernatant containing the released adenine nucleotides the same volume of 1.2 mol/l perchloric acid was added. Samples were kept at 4°C and then centrifuged again (9). The amount of released adenine nucleotides in supernatant was determined spectrophotometrically and expressed as the percent of their total amount in the cells (3, 11).

Platelet aggregation: Platelet aggregation was measured by turbidimetric method of Born (12). ADP-induced aggregation of control platelets and platelets after bed rest was registered in an aggregometer using 10 μM ADP as an aggregatory agent.

Statistical analysis was performed by Students't test for paired data.

RESULTS

The level of total adenine nucleotides in control platelets was 312.1± 35.4 nmol/10^{10} platelets *(Fig. 1)*. Long term bed rest significantly ($p < 0.05$) affected platelet adenine nucleotides concentration. Contrary to 14 days lasting bed rest, when only slight changes in the level of adenine nucleotides were observed, after 28 days bed rest the concentration of platelet adenine nucleotides was considerably reduced (by about 30%) and the loss of ATP and ADP (about 30% and 50%, respectively), occured *(Fig. 1)*.

Thrombin released 58.2%±1.5% of total adenine nucleotides from platelets of control individuals and 50.8%±4.7% from patients after 14 days of bed rest *(Table 1, Fig. 2)*. 4 weeks lasting immobilization in bed caused release of decreased amount of nucleotides from platelets ($p < 0.05$) even at high

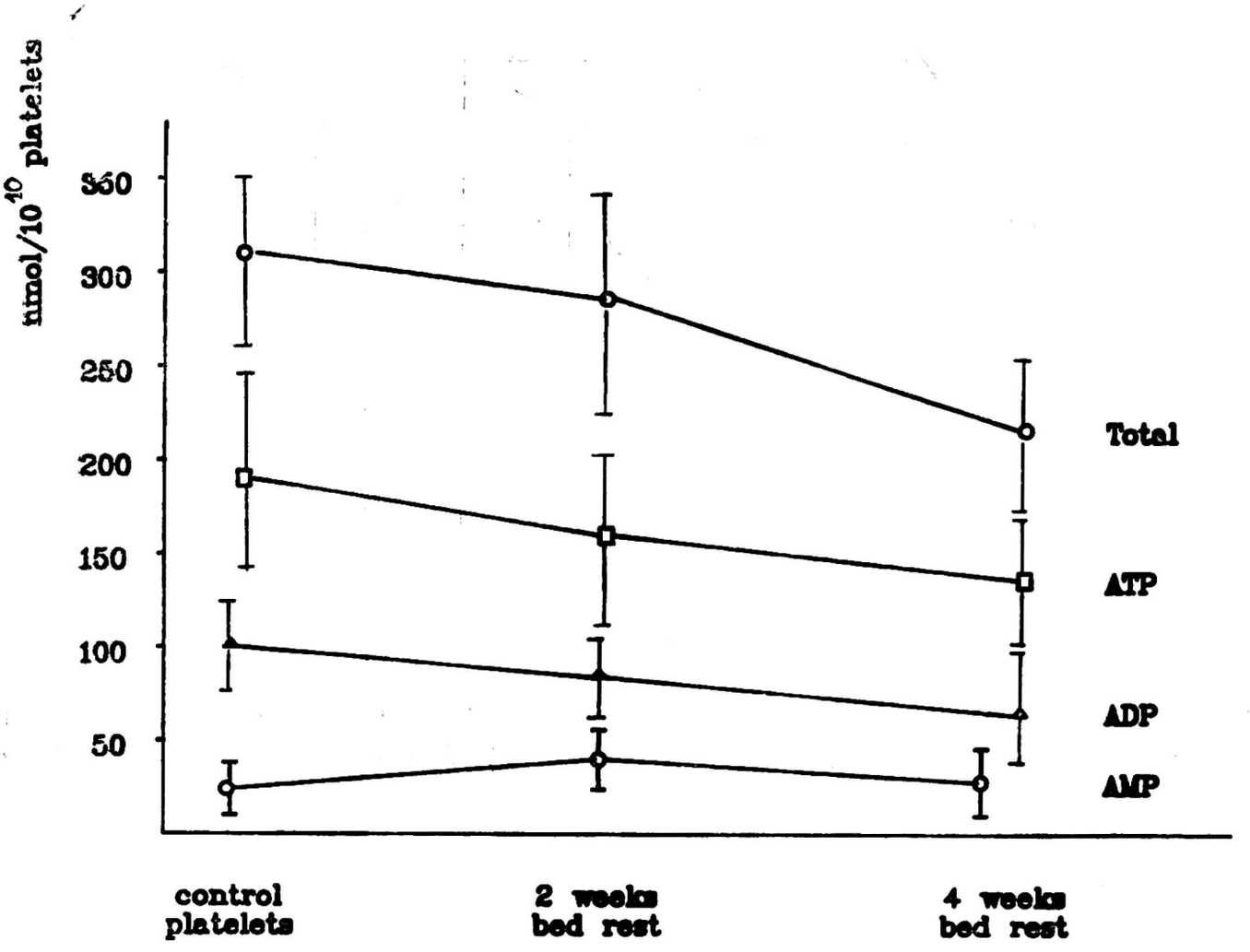

Fig. 1. The effect of bed rest on the adenine nucleotides concentration in human blood platelets. Values (nmol/10^{10} platelets) are mean ±SD; n = 15; $p < 0.05$

Table 1.

Thrombin – induced release of adenine nucleotides from control platelets and from platelets of patients immobilized in bed. Values are expressed as % of total platelet adenine nucleotides. (n=11, p<0.05)

	Thrombin – released adenine nucleotides (% of total)		
	control platelets	2 weeks bed rest	4 weeks bed rest
1	57.5	45.5	26.2
2	59.0	45.8	27.2
3	58.3	47.1	29.2
4	56.7	46.2	18.3
5	57.9	56.0	19.8
6	59.2	55.1	21.4
7	58.7	54.3	20.5
8	60.5	48.9	22.1
9	55.5	49.8	21.7
10	59.9	50.7	23.2
11	56.5	59.5	24.5
$\bar{x}$	58.2	50.8	23.1
±SD	±1.5	±4.7	±3.3

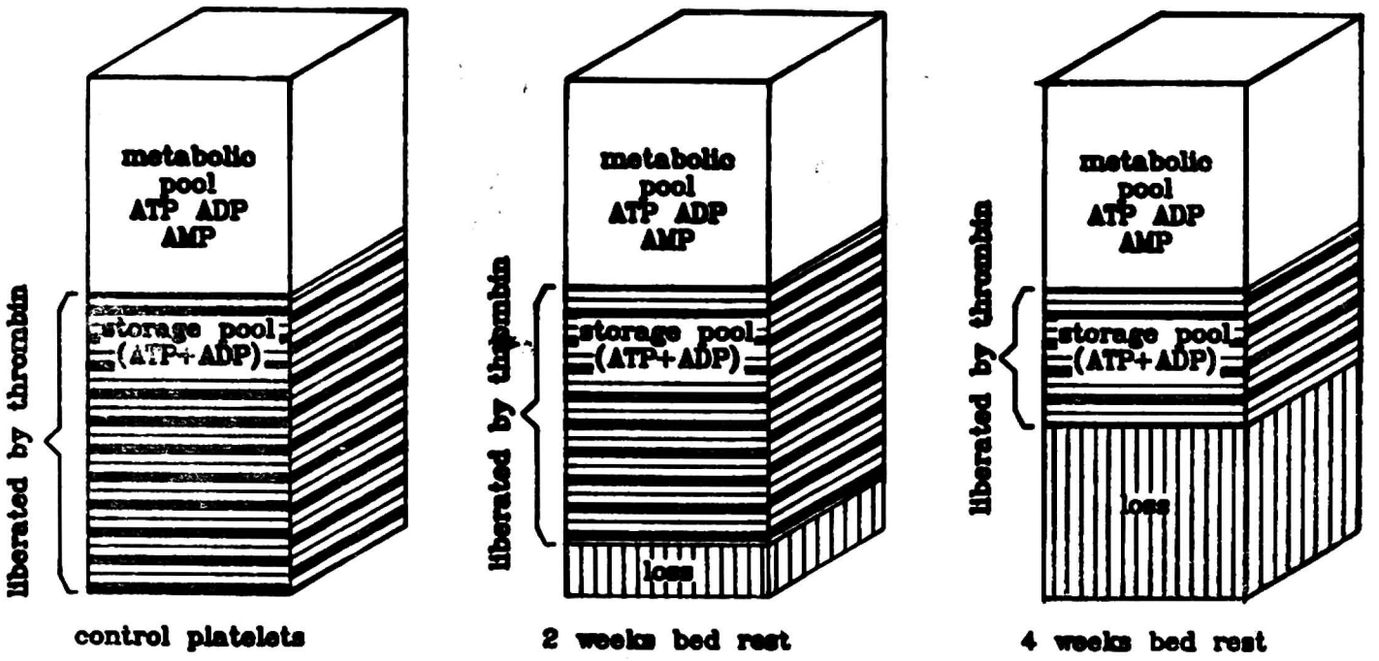

Fig. 2. Compartmentation of adenine nucleotides in control blood platelets and in platelets of patients after bed rest. n = 11. Values are expressed as % of total platelet adenine nucleotides ($p < 0.05$)

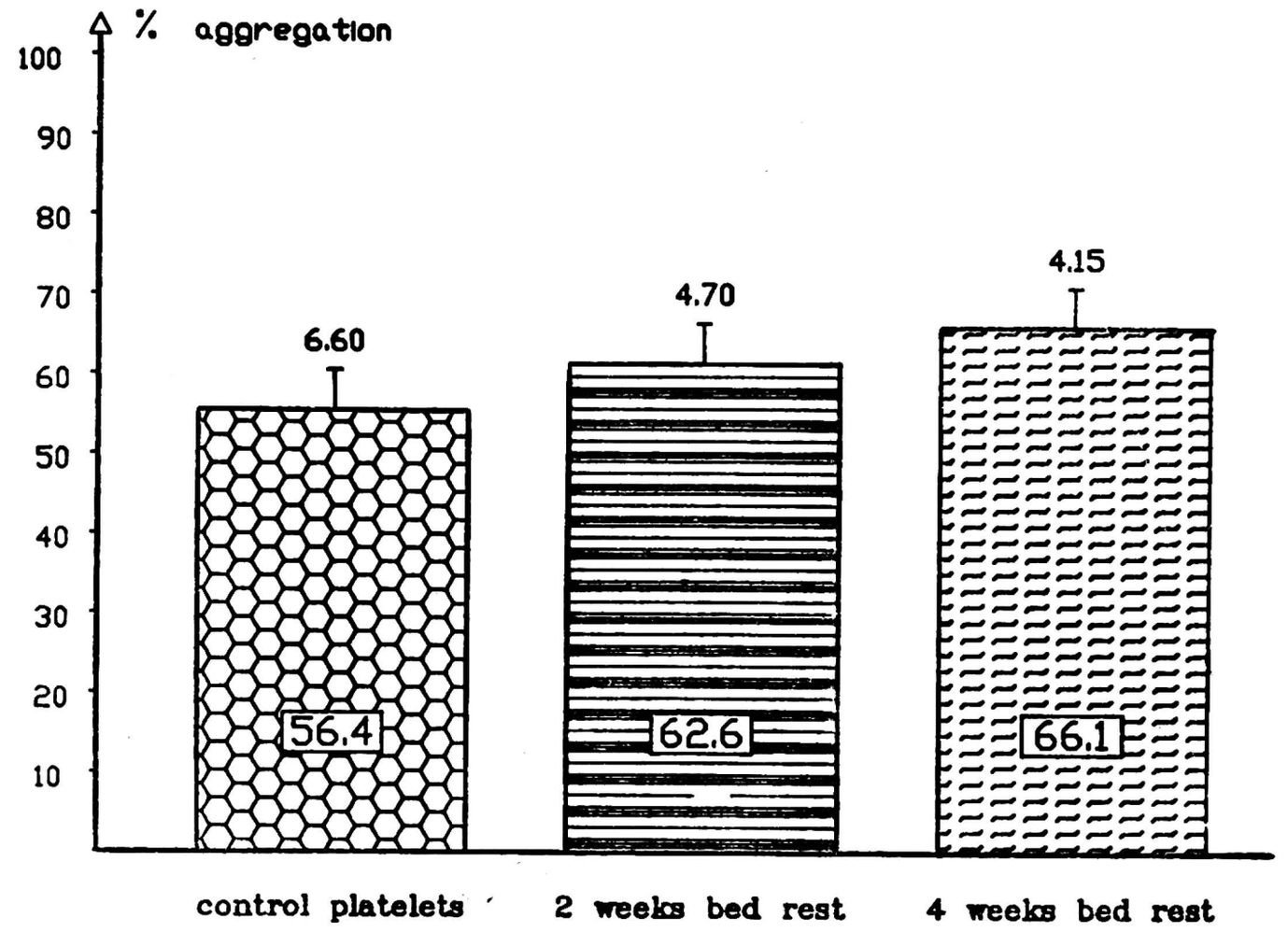

Fig. 3. 10 μM ADP-induced platelet aggregation (%) before and after 2 weeks and 4 weeks bed rest. Value given are mean ±SD; n = 15; $p < 0.05$. To 1 ml of platelet rich plasma containing 3.5×10^8 platelets ADP was added.

concentration of thrombin (10 units) and after 4 weeks immobilization in bed thrombin was able to liberate only 23.1%±3.3% of total adenine nucleotides present in the cell *(Fig. 2, Table 1)*.

It was found that 2 weeks and 4 weeks immobilization in bed increased the extent of ADP-induced platelet aggregation about 11.1% and 11.7%, respectively *(Fig. 3)*.

DISCUSSION

During bed rest many physiological and biochemical changes, particularly in cardiovascular and excretory systems occur (13—15). The pathological processes in blood platelets after long term bed rest are unknown. We observed that blood platelets of patients after immobilization in bed differ significantly in their behaviour from control platelets and this difference can be caused by hyperactivity of these cells.

The results of our study indicate that long term bed rest (4 weeks) leads to stimulation of platelet activation-release of adenine nucleotides *(Fig. 1)* and increased aggregation of these cells *(Fig. 3)*. Following 4 weeks bed rest, the loss of the total adenine nucleotides (mainly ADP and ATP) seems to be caused by the secretory process but not by damage to the cells. Specific secretory process (platelet release reaction) is induced by many agents and it leads to the release of about 60% of total (ADP and ATP) platelet adenine nucleotides (1, 3). We found that in case of short term bed rest, thrombin releases slightly reduced amount of nucleotides (50.8% of total instead of 58.2%). After 4 weeks immobilization in bed thrombin liberates only 23.1% of total nucleotides and it is not able to release more. It indicates that during bed rest the secretory process starts and the loss of adenine nucleotides from bloood platelets after 4 weeks immobilization in bed is due to the release of these compounds from the platelet storage pool. Therefore, thrombin is able to liberate only the rest of storaged nucleotides *Fig. 2*. The secretory process in blood platelets observed after bed rest is accompanied by increased ($p < 0.05$) platelet aggregation *(Fig. 3)*.

In blood platelets after 2 weeks of bed rest the amount of ATP is slightly reduced and this decrease correlates partly with an increase of platelet AMP *(Fig. 1)*: it suggests that these changes may be dependent also on the alteration in the platelet adenine metabolic pool. It should be pointed out that there is a correlation between the level of metabolic ATP and the ability of the platelet to perform aggregation and release reaction (9, 10).

Long lasting immobilization caused by bone fractures seems to lead to "acquired storage pool disease". This syndrom is probably dependent on the increased thrombin generation observed in veins of immobilized patients.

Activation of blood platelets (adhesion, aggregation, release reaction) plays an important role in the initiation of the blood clotting process. The stimulating effect of immobilization in bed on the release of adenine nucleotides from platelets and the increased extent of platelet aggregation indicates that bed rest may cause the hyperactivity of platelets and the stimulation of blood clotting process.

At present no data are available concerning the prolonged immobilization effect on blood platelet behaviour. It is concluded that immobilization in bed resulting from the contemporary model of life, leads to many changes in metabolism and function of blood platelets. The effect of bone fractures on blood platelets behaviour after long term bed rest should be neglected because the life time of these cells amounts about two weeks; the same opinion present Ernst et al. (14).

REFERENCES

1. Fukami MH, Holmsen H, Salganicoff L. Adenine nucleotides metabolism of blood platelets. IX. Time course of secretion and changes of energy metabolism in thrombin-treated platelets. *Biochim Biophys Acta* 1976; 74: 301—305.
2. Holmsen H. Energy metabolism and platelet responses. *Vox Sang* 1981; 40: 1—7.
3. Holmsen H. Day HJ. Adenine nucleotides and platelet function. *Ser Haematol* 1971; 4: 28—58.
4. Tkaczewski W, Kędziora J, Buczyński A, Ryniec A, Błaszczyk J, Dziekański S. Blood platelets: aggregation, malonyldialdehyde concentration and SOD-1 activity in patients with coronary heart disease after captopril administration. In: *Current Advances in ACE Inhibition*. A Mac Gregor, PS Sever (eds). Churchill Livingstone 1989; pp. 191—193.
5. Buczyński A, Kędziora J. Effect of submaximal physical exercise on oxygen metabolism in blood platelets of healthy men. XXXI *Intern Congress Physiol Sciences*, Helsinki, 1989.
6. Kędziora J, Buczyński A, Kafar K, Żołyński K. Effect of hypokinesis on antioxidative defense in blood platelets. *Acta Physiol Pol.* 1990; 34; 161—162.
7. Buczyński A, Kędziora J, Tkaczewski W, Wachowicz B. Effect of submaximal physical exercise on antioxidative protection of human blood platelets. *Intern J Sport Med* 1991; 12: 52—54.
8. Błaszczyk J, Buczyński A, Kędziora J. Blood platelet and erythrocyte superoxide dismutase (SOD-1) activity and malonyldialdehyde concentration in healthy men following submaximal exercise. *Acta Physiol Pol* 1987; 38: 56—57.
9. Wachowicz B. Adenine nucleotides in thrombocytes of birds. *Cell Biochem Funct* 1984; 2: 167—170.
10. Wachowicz B, Krajewski T. Activation of blood platelets and prostaglandin biosynthesis. *Acta Universitatis Lodz.* 1986; 5: 133—143.
11. Wachowicz B, Buczyński A, Krajewski T, Tkaczewski W, Kędziora J. Microtechnique of adenine nucleotides assay in human platelets. *Pol Tyg Lek* 1988; 7: 230—233.
12. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 1962; 194: 927—928.
13. Birkhead NC, Haupt GJ, Mayers RN. Effect of prolonged bed rest on cardio-dynamics. *Am J Med Sci* 1963; 245: 118—119.

14. Ernst E, Schmidt-Pauly E, Muhling P, Matrai A. Blood viscosity in patients with bone fractures and long term bed rest. *Br J Surg* 1987; 74: 301—305.
15. Greenleaf JE, Bernauer EM, Juhos LT, Young HL, Morse JT, Staley RW. Effects of exercise on fluid exchange and body composition in man during 14 days bed rest. *J Appl Physiol Respirat Environ Exercise Physiol* 1977; 43: 126—132.

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