The Release of Platelet Factor 4 During Platelet Aggregation and the Possible Significance of this Reaction in Hemostasis

Platelet factor 4 (PF_4) was originally described as an antiheparin factor[1]. NIEWIAROWSKI et al.[2] demonstrated that this factor, obtained from pig platelets in a purified form, neutralizes the anticlotting activities of fibrinogen breakdown products (antithrombin VI). Further investigations[3] have shown that purified PF_4 is a potent paracoagulating agent. It induces, as does protamine sulphate (PS), non-enzymatic clotting of soluble fibrin monomers complexes with fibrin degradation products. These complexes are intermediate products of fibrin clot proteolysis. A method for the determination of PF_4 based upon its paracoagulating activity, has also been described. Finally it has been observed that both the antiheparin and paracoagulating activities of PF_4 are released during aggregation, induced by ADP, thrombin, collagen, serotonin and adrenaline[3].

Moreover, we have investigated the kinetics of the release of PF_4 during platelet aggregation by ADP and adrenaline (Figures 1 and 2). The experiments were performed by adding 3.6 ml of platelet-rich plasma (PRP) and 0.4 ml of the aggregating agent to the photometer cell. The mixture was constantly stirred. The decrease in optical density (O.D.)[4] and increase in the antiheparin activity[5] were recorded at the same time. It can be seen that the release of PF_4 by ADP and by higher adrenaline concentrations occurs at the moment of visible aggregate formation. The final amounts of PF_4 released depends upon the dose of ADP or adrenaline. It can be noticed that small concentrations of adrenaline ($10^{-7}M$) do not release significant amounts of PF_4 while producing fine platelet aggregates.

PF_4, released during aggregation, is not bound to platelet membranes and it is completely recovered in the supernatant plasma after centrifugation of aggregated platelets. The Table shows a correlation between the antiheparin and paracoagulating activities tested in the supernatant plasma after platelet aggregation.

It is thought that PF_4 may play a double role in the mechanism of platelet aggregation.

The release of PF_4, which seems to be a basic protein, may change the surface properties of platelet membranes. This fact may contribute to the complex mechanism of platelet aggregation and to the modification of the electrokinetic response of platelets to aggregation agents[6]. PF_4 is released in all types of aggregation except those induced by small concentrations of adrenaline. The latter type of aggregation has also other peculiar features: there is no release of ADP[7] and no change of platelet shape or of platelet volume[8].

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Release of PF_4 as measured in heparin – thrombin time and in paracoagulation tests[a]

Substance added to PRP	Aggregation rate decrease in O.D. after 5 min	Heparin thrombin time, sec	Paracoagulation O.D. units
Saline	0.00	32	0
ADP $10^{-6}M$	0.50	15	27
$2 \times 10^{-7}M$	0.40	18	20
$2 \times 10^{-9}M$	0.02	30	7
Adrenaline			
$2 \times 10^{-5}M$	0.75	15	33
$2 \times 10^{-6}M$	0.63	20	27
$2 \times 10^{-7}M$	0.50	22	15
$2 \times 10^{-8}M$	0.38	30	4

[a] To test the paracoagulating activity of PF_4 in plasma, PRP was centrifuged to remove platelets, heated at 60 °C for 10 min and dialyzed overnight against saline to remove citrate. The test was performed by mixing 0.1 ml of the dialyzate with 0.4 ml of fibrin lysate. O.D. changes were recorded after 2 min. Details of the method will be reported elsewhere[3].

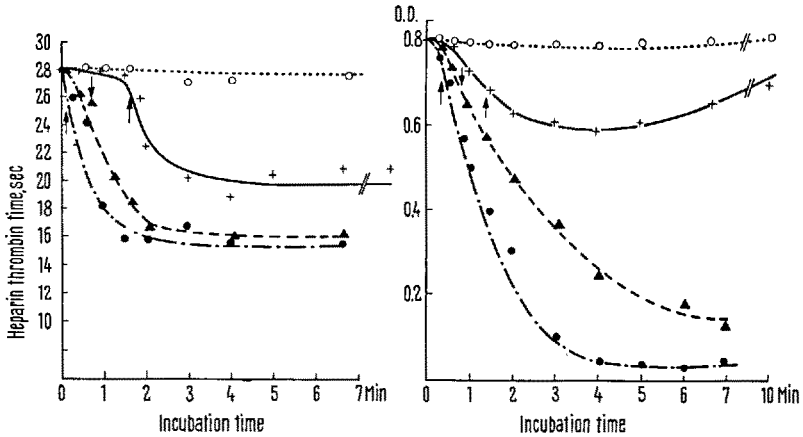

Fig. 1. Release of PF_4 during platelet aggregation by ADP (aggregation indicated by the arrows). o····o PRP + saline, +——+ PRP + ADP $3 \times 10^{-7}M$, ▲----▲ PRP + ADP $\times 10^{-6}M$, ●——● PRP + ADP $\times 10^{-5}M$.

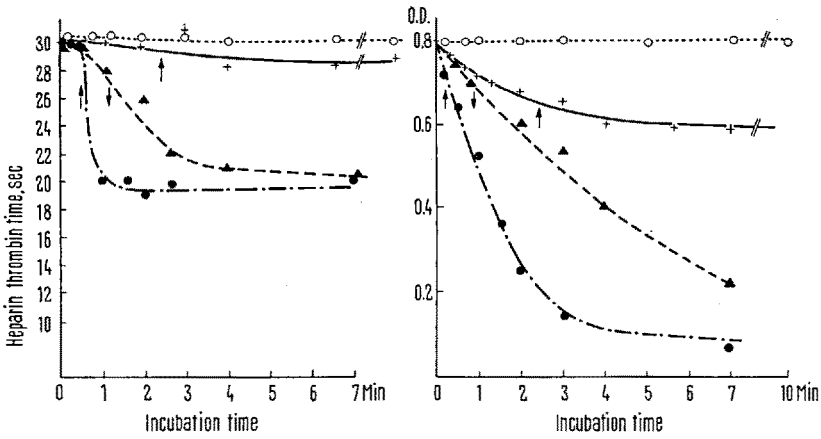

Fig. 2. Release of PF_4 during platelet aggregation by adrenaline (aggregation indicated by the arrows). o----o PRP + saline, +——+ PRP + adrenaline $10^{-7}M$, ▲----▲ PRP + adrenaline $10^{-6}M$, ●——● PRP + adrenaline $10^{-4}M$.

The second possible explanation is that PF_4 may induce paracoagulation of soluble fibrin monomer complexes occurring in the platelet atmosphere. Formation of fibrin threads between platelets may cause their mutual attachment. In support of these data we may quote microscopic observations showing platelets as centres of fibrin thread formation in the hemostatic plug, and SOLUM's findings[9] on the induction of platelet aggregation by fibrin oligomers.

Résumé. Nous avons constaté que le facteur plaquettaire 4 est libéré au début de l'aggrégation des plaquettes par l'ADP et l'adrénaline. Ce phénomène a pu être mis en évidence en étudiant l'activité antihéparinique et l'activité paracoagulante (coagulation non enzymatique de complexes solubles de monomères de fibrine) du facteur plaquettaire 4.

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Photosynthesis and Respiration II. Effect of 3-(3,4-Dichlorophenyl)-1,1-Dimethylurea and of Partial Pressure of Oxygen on the Rates of Carbon Dioxide Exchange in Light and in Darkness of Detached Wheat Leaves

In our previous work[1], we found entirely different effects of various metabolic inhibitors on the rates of CO_2 evolution in light and in darkness. It is known[2] that CO_2 evolution in light or photorespiration is greatly dependent on the oxygen concentration in ambient air (stimulation) whereas dark respiration was practically unaffected by an oxygen concentration as low as 1%.

The present study was designed to investigate the simultaneous action of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and of partial pressure of oxygen on the rates of CO_2 exchange in light and in darkness of detached wheat leaves. DCMU is an extremely specific inhibitor of photosynthesis[3] which does not interfere with the dark respiration or fermentation. Therefore, using the DCMU, one can expect that after suspension of CO_2 uptake in light, the rates of CO_2 evolution in light or in darkness must be similar if these 2 processes are identical. Moreover, using oxygen as a stimulating factor of CO_2 output in light, we can observe the effect of partial pressure of oxygen on respiration both in light and in darkness in the absence of photosynthesis.

Detached wheat leaves (*Triticum vulgare* Vill. var. Thatcher) taken from 1-month-old plants were used as experimental material. Measuring the carbon dioxide exchange rates both in light and in darkness, the application of DCMU to the leaves and other details of the methods used in the present study were identical to those described in our previous publication[1].

The Table shows the simultaneous effect of 10^{-5} and $10^{-6}M$ of DCMU and 1 and 100% of oxygen on the rates of CO_2 exchange of wheat leaves. The rates of apparent photosynthesis (APS) in an atmosphere of 100% oxygen were about 80% lower as compared with those in 1% oxygen. The rates of dark respiration in both atmospheres of oxygen were similar. However, the rates of photorespiration (PR) were very low in 1% O_2 and were about 30 times higher in 100% O_2. DCMU in applied concentrations inhibited the rates of apparent photosynthesis in an atmosphere of 1% O_2 by about 90% as compared with those in control, and completely in the pure oxygen. The rates of dark respiration (DR) were practically the same in the presence or absense of DCMU. After inhibition of

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