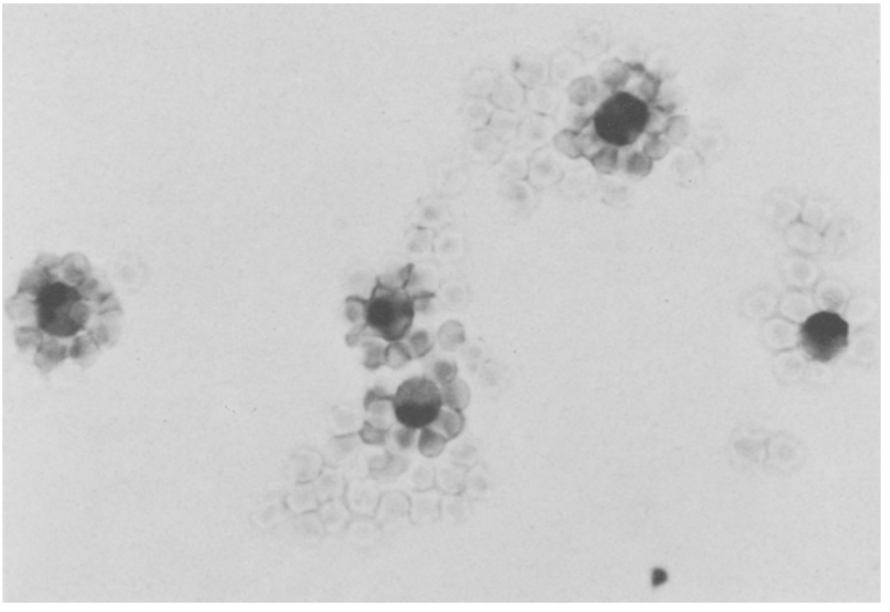

E-rosettes formed by ANAE positive lymphocytes showing the typical dot-like reaction product.

not form E-rosettes. One explanation would be that some E-rosettes have been disrupted by the cytocentrifugation. The determination of the percentage of E-rosette forming cells in 22 experiments before (72±9.3%) and after (73±7.7%) cytocentrifugation, however, makes this explanation unlikely. Another possibility could be the staining time of 16 h at 4 °C which we used, compared with 3 h at 37 °C prefered by other authors. Comparing these different methods, the percentage of 'localized' cells is the same, changes occurring mainly among the 'granular' and 'negative' subpopulations[4].

It is concluded that there is a loose but not definite correlation between the ANAE staining pattern and E-rosette formation capability of the lymphocytes. The ANAE-assay allows one clearly to identify different lymphocyte subpopulations, and the combined rosette forming/ANAE technique might help to define in further detail origin and functional activity of lymphocytes.

Current experiments are based upon the hypothesis that lymphocytes with a localized, dot-like reaction product are resting mature T cells, whereas cells with a different staining pattern may be B cells, activated T cells or thymocytes. It remains to be clarified why lymphocytes change their enzyme activity during their ontogenesis and in different states of activation[5].

According to current knowledge the ANAE technique is not a method to replace but to supplement more conventional techniques of lymphocyte differentiation. Further evaluation of the clinical relevance of the ANAE activity is required.

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Platelet aggregation following electrical stimulation

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Summary. It was demonstrated that the previous in vitro electrical stimulation of human and rat platelet-rich plasma does not modify the subsequent response of platelets to the aggregating activity of ADP, thrombin, thrombofax or adrenaline. This is interesting in view of the fact that the electrical stimulation can induce clot retraction.

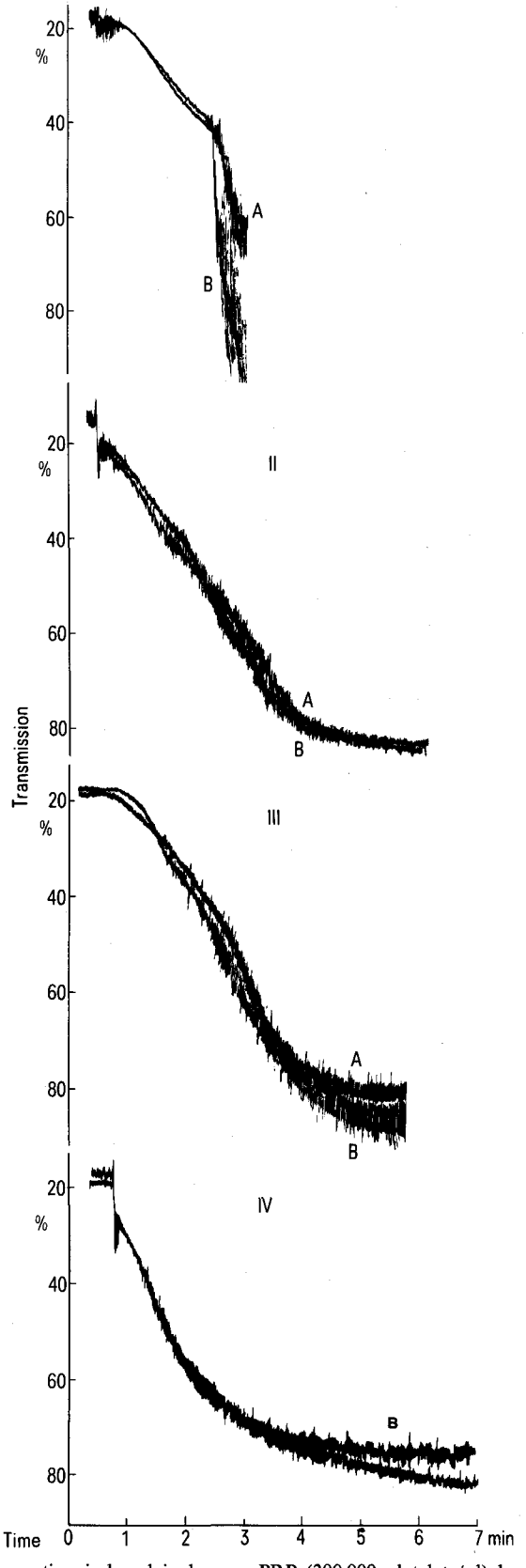
Recent work from our laboratory has demonstrated that it is possible to induce clot retraction by electrical stimulation of platelet-rich plasma clotted by reptilase[2,3]. The retraction is due to platelet activation, as it is absent in platelet-poor plasma; and it is inhibited by several inhibitors of platelet functions[2,3].

Several papers have demonstrated that many conditions influencing clot retraction can also modify the platelet adhesion-aggregation reaction[4-7]; therefore it seemed interesting to verify the influence of the electrical stimulation on the platelet adhesion-aggregation reaction.

The present paper shows that the previous in vitro electrical stimulation of platelet-rich plasma is ineffective on the platelet aggregation induced by ADP, adrenaline, thrombin and thrombofax.

Materials and methods. Human and rat venous blood was collected as previously described[8,9]. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) and platelet count were performed as previously described[9].

Platelet aggregation was tested in PRP with standard platelet concentration (700,000/µl in rat PRP; 300,000/µl in human PRP), by an aggregometer (169 Platelet Aggrega-



Aggregation induced in human PRP (300,000 platelets/µl) by: I, thrombin (final concentration 0.5 U/ml); II, adrenaline (final concentration 10 µg/ml); III, thrombofax (final dilution 1:5); IV, ADP (final dilution (2×10^{-5} M), before (A) and after (B) an electrical stimulation (intensity 150 V; frequency 10/sec; duration of each stimulus 50 msec) prolonged for 60 min.

tion Meter, Evans Electroselenium Ltd) and recorded by Speedomax XL 690 Series Recorder (Lees and Northrup, North Wales and Philadelphia) as usual[9].

Electrical stimulation of PRP was performed at room temperature by means of 2 platinum electrodes (2 mm length; 0.5 mm diameter) connected to a stimulator (S 8 Stimulator, Grass instruments, Quincy, Ma. USA). The electrodes were immersed one at the top and the other at the bottom of the PRP (about 10 ml) contained in a plastic tube (internal diameter 12 mm). The stimulation (intensity: from 10 to 150 V; frequency: from 2 to 15/sec; duration of each stimulus: from 10 to 75 msec) was prolonged for 60 min. At regular intervals of time a sample of PRP was removed with a pipette and platelet aggregation was tested.

Chemicals used are: adenosine-5'-diphosphate (ADP) trisodium salt (Boehringer, Mannheim, Germany) dissolved in buffered saline pH 6.8 at a concentration of 10^{-3} M and stored at −20 °C until use; adrenaline cristals (Merck, Darmstadt, Germany); thrombofax (Ortho diagnostic, Raritan, N.J. USA); thrombin (Topostasine Roche, Milano, Italy).

Results. The previous in vitro electrical stimulation of PRP (intensity: 150 V; frequency: 10/sec; duration of each stimulus: 50 msec) does not modify at all the subsequent response of platelets to the aggregating activity of ADP (5 experiments), adrenaline (5 experiments), thrombin (5 experiments) or thrombofax (5 experiments) in human PRP (figure).

Similar results were constantly found also in rat PRP where, however, thrombofax and adrenaline were not tested. Quite similar results were obtained following electrical stimulation performed under variable intensity (10–150 V), frequency (2–15/sec), duration (10–75 msec for each stimulus).

Therefore it appears clearly that the electrical stimulation of PRP (even prolonged for 60 min) does not modify the subsequent aggregation of platelet induced by ADP, adrenaline, thrombofax or thrombin.

Discussion. Previous papers showed that many conditions able to influence clot-retraction can also modify the platelet adhesion aggregation reaction[4–7]. Present results show that electrical stimulation fails to modify platelet aggregation, even if it can induce the reptilase-clot retraction[2,3]. Therefore it may be necessary to reconsider the previously formulated hypothesis[4–7] that clot retraction is simply due to the activation of the same contractile system involved in the platelet adhesion aggregation reaction.

However we cannot exclude that electrical stimulation has a different efficacy on platelets suspended in PRP or immobilized within the clot only because of the different physical characteristics of the surroundings around platelets. This could be the only reason why even a very prolonged electrical stimulation of PRP is ineffective in modifying platelet aggregation.

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