plasma membrane. Intracytoplasmic type A particles were occasionally seen. Several P388 cells were observed to have dense aggregates of type A particles in their cytoplasm (figure, D). In a few instances immature type B particles or apparent budding of type A particles were seen (figure, E). Cell-free particulate material was harvested from the supernatant of another 48 h P388 culture by differential centrifugation, exposed to 1% glutaraldehyde in a tris-sodium chloride-ethylenediaminetetraacetic acid buffer[6], and then subjected to negative staining with 1% potassium phosphotungstate. Numerous leukemia virus-like particles, lacking distinctive peplomers or spikes, were observed (figure, F). Type C particles seem to be the predominant oncornavirus-like entities produced by cultured P388 cells. The production of type C particles is apparently a common event in the cultured P388 cells to be so readily detectible by the relatively insensitive electron microscopy approach. Further experiments to assay in vitro infectivity titers of P388 cells and their type C particles in other cultured cells will be undertaken.

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Identification of lymphocyte subpopulations by simultaneous E-rosette formation and unspecific acid esterase staining

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Summary. E-rosettes of human peripheral blood lymphocytes were stained by an unspecific acid esterase stain; 74.5% of the rosette forming cells were esterase positive, while only 30% of the non-rosette forming cells showed a reaction product.

There is accumulating evidence that lymphocytes presenting with localized acid alpha-naphthyl-acetate-esterase (ANAE) activity are T cells. ANAE positive lymphocytes are found mainly in the cortical areas of the mouse lymph node, but not in the germinal centres[1]. Removal of E-rosette forming lymphocytes by Ficoll-Hypaque centrifugation results in a marked depletion of ANAE positive cells in the supernatant[2]. 85% of peripheral human blood lymphocytes and 90% of tonsillar T lymphocytes are ANAE positive, whereas only 12% of isolated B cells are positive[3]. Such cells are characterized by a strong, well-defined and dot-like reaction product, in contrast to human thymocytes, of which only about 30% are ANAE positive, with a faint cytoplasmatic staining of variable intensity. These different staining patterns are described as T-like and Thy-like, as opposed to the well-known intense and diffuse reaction product in monocytes (M-like)[3]. We report further evidence that esterase positive cells are of T cell origin by direct esterase-staining of rosette forming cells.

Methods. Peripheral blood lymphocytes were obtained from 19 healthy volunteers (age range 29–50 years, median 35 years). Ficoll-Ronpacon density gradient isolated lymphocytes were incubated in 0.83% NH_4Cl at 37 °C for 15 min to destroy remaining erythrocytes, washed 3 times with MEM and resuspended in MEM at a concentration of 10×10^6 cells/ml.

Sheep blood was diluted 1:2 with Alsevers solution. Aliquots were washed 3 times with 0.9% NaCl and resuspended at a final concentration of 80×10^6 sheep red blood cells (SRBC)/ml. Anti-SRBC antibodies were removed by incubation of SRBC in bovine serum for 30 min at 4 °C and at 37 °C. 0.05 ml of SRBC free bovine serum and 0.05 ml of the resuspended SRBC were added to 0.1 ml of human peripheral blood lymphocytes. This mixture was centifuged at $200 \times g$ for 5 min, gently resuspended and rosettes (≥ 3 SRBC/lymphocyte) counted in an aliquot of this suspension. Thereafter the rosettes were fixed with 5% glutaraldehyde in saline. Smears were prepared by cytocentrifuge sedimentation and immediately stained for 16 h at 4 °C by the method described by Müller[1] using alpha-naphthyl-acetate as substrate and hexazotized pararosanilin as coupler. We defined as 'positive' all lymphocytes showing some kind of reaction product.

Subgroups of the 'positive' population were classified as 'localized': cells displaying a strong, dot-like staining pattern with 1 or 2 dots (T-like); as 'granular': cells displaying a more diffuse, fine granular staining pattern in the cytoplasm (Thy-like).

Cells were called 'negative' if they did not show any evidence of ANAE activity.

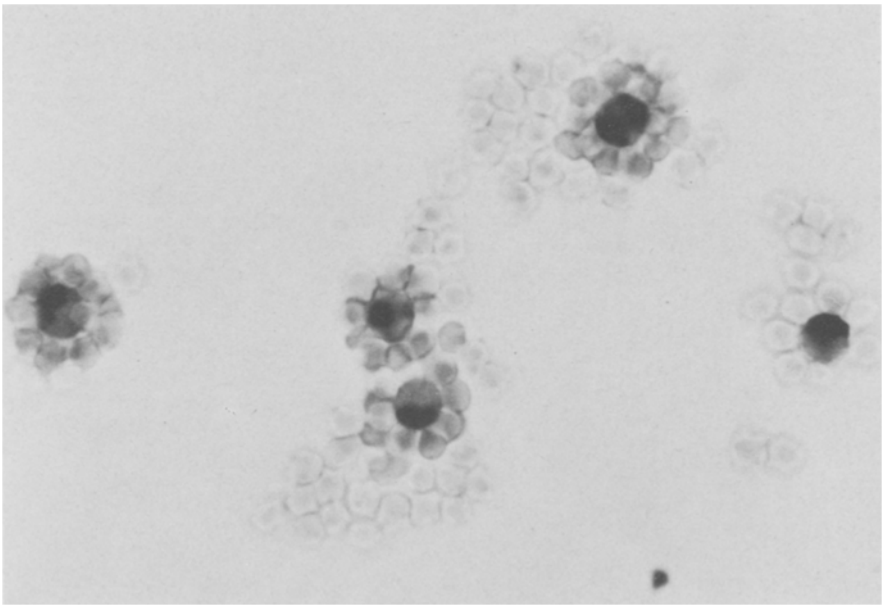
Results and discussion. In preliminary experiments we had determined in parallel the percentage of ANAE positive and E-rosette forming cells in the same blood sample of 25 healthy volunteers: $94 \pm 4.2\%$ of the blood lymphocytes were ANAE positive and $75 \pm 4.7\%$ formed E-rosettes.

In these series we directly stained E-rosettes as shown in the figure. The intense dot-like reaction product can be seen clearly, the characteristic localization being at the periphery of the cell.

The results are shown in the table: 74.5% of the ANAE positive cells formed E-rosettes, 25.5% did not. Of the 'localized' subgroup 76%, of the 'granular' subgroup 66%, formed E-rosettes, whereas only 30% of the ANAE negative lymphocytes did so. These results add further evidence that ANAE positive cells are in fact T cells. The question remains, however, why 25.5% of ANAE positive cells did

Percentages of E-rosette positive and negative lymphocytes among the esterase subpopulations

	E-rosettes Positive (73%)	Negative (27%)
Esterase positive	74.5± 8.8	25.5± 8.8
Localized	76 ± 8.1	24 ± 8.1
Granular	66 ±24.9	34 ±24.9
Esterase negative	30 ±20.4	70 ±20.4



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-