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STORAGE OF LEUKCCYTE POOR PLATELET CONCENTRATES PREPARED FROM BUFFYCOATS IN VARIOUS TYPES OF PLASTIC BAG
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Platelet concentrates (PC) prepared from buffycoats in a quadruple bag CPD-SAG M system proved to have a good survival in vivo after storage for 72 h at 22°C (Vox Sang 1985, 49: 81-85). Storage of these PC for 7 days at 22°C on a linear platelet reciprocator, was studied using 6 different types of bag made of polyvinylchloride (PVC) with either DEHP and/or TOTM as plasticizer. From Biotest the 76 standard PVC (n=14) and 763 thin PVC (n=16)bag, from NPBI the PSV 3277 standard PVC (n=15) and DPL-110 TOTM (n=14) and from Terumo the old (n=18) and new (n=14) molded Teruflex bags were examined.
The Biotest 763, the NPBI DPL-110 and the Terumo new molded Teruflex are recommended for platelet storage.
The plasma volumes averaged 63 ml with a range of 39 to 81 ml. Platelet concentrations were comparable in all types of bag with an overall mean of 0.89±0.33 (SD)x10^9/ml. The number of contaminating leukocytes and erythrocytes never exceeded 10^7 per unit. The initial pH was between 6.98 and 7.19. After 7 days storage the pH in all bags remained above the critical value of 6.0 with a range of 6.56 to 7.38. No decrease of PO_2 was observed during storage, on the contrary a significant ($p<0.05$) increase in the PSV 3277 and new molded Teruflex bags was measured. The PCO_2 diminished equally in all bags also indicating adequate gas exchange. Good morphology scores of the platelets (greater than 200) were observed in 98% of the PC after 5 day storage and in 83% after 7 days.
These data indicate that PC can be equally well be stored in normal PVC bags as in special platelet storage bags, provided that the PC are leukocyte poor.

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CONTAMINATING LEUKOCYTES INFLUENCE THE STORAGE CONDITIONS OF PLATELET CONCENTRATES
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Leukocyte poor platelet concentrates (PC), containing less than 10^7 leukocytes, prepared from buffycoats can be stored in normal PVC bags for 7 days at 22°C without deterioration of the pH. We assumed that a low number of leukocytes present in the PC, is a critical factor to maintain the pH. To test this hypothesis increasing amounts of leukocytes were added to four groups of three PC with comparable plasma volumes (mean 58.6± 0.8 (SD) ml) and platelet concentrations (1.01±0.04x10^9/ml). Group I had a leukocyte concentration of 0.14±0.048x10^6/ml, group II 1.96±0.09x10^6/ml, group III 5.53±0.98x10^6/ml, and group IV 13.0±0.93x10^6/ml. The PC were stored in normal PVC bags for 7 days at 22°C. Measurements in vitro were performed at day 0, 2, 5 and 7.
The initial mean pH value was 7.12±0.02 (SD) for all PC and dropped to 6.89, 6.85, 6.77 and 6.61 for group I to IV respectively, at day 7. A significant correlation (Spearman rank test) between low pH values and high leukocytes was found. The same significant positive correlation was observed between high leukocyte concentrations and high glucose consumption and high lactate production and LDH release during storage.
These results show that the amount of leukocytes in PC has a significant contribution to the detrimental effect on pH during platelet storage. It is therefore important to prepare PC with a leukocyte count lower than 10^7. Moreover the risk of alloimmunisation against HLA antigens will be diminished.

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EFFECTS OF PLASMA MEMBRANE GLYCOPROTEIN OF PLATELETS ON THE AGGREGATION ACTIVITY OF THE STORED CELLS.
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Platelet aggregation activity is gradually reduced depending on the duration of storage. Platelet membrane glycoprotein IIb/IIIa (GPIIb/IIIa) complex is intimately related to the aggregation activity. We intended, therefore, to analyse a sequential change in plasma membrane glycoprotein of stored platelets by means of flow cytometry (FCM). On this occasion, aggregation activity of the platelets was also studied. Concentrated human platelets (150x10^4 cell/µl) were stored in the bag containing citrate-phosphate-dextrose at 22 °C for 24 to 72 hours. The bags were either kept in a flat position without agitation or continuosly stirred by a tumbler agitator (6rpm), or a flat bed rotator (30rpm). At the beginning of each experiment, fresh platelets separated from healthy donor were used as control group. The remaining platelets were washed twice with the 0.38% sodium-citrate dissolved in the 10mM of PBS. After incubation of the suspended platelets in the Tyrode's buffer solution at 37 °C for 30 minutes, they were fixed with 1% paraformaldehyde at 4 C for 2 hours, and were then incubated with anti-human GP IIb/IIIa mouse monoclonal antibody (5µg/ml) at 37 °C for 1 hour. The platelets, thus treated with primary antibody, had undergone further incubation with fluorescein-conjugated goat anti-mouse IgG immunoglobulin at 37 °C for 1 hour. After analysis of labelled platelets on the Coulter EPICS V, the positive rate was estimated by counting 5x10^4 cells. In each group, aggregation activity of platelets induced by ADP (100µM) was measured by an aggregometer.

The positive rate was significantly decreased in the stored platelets compared with those in the control group. In addition, the positive rate was more decreased in the non-agitated group than in the agitated group. No difference, however, occurred in the rate from the agitated groups. Moreover, the aggregation activity in each group was well compatible with the positive rate from FCM.

It is finally suggested that the decrease in aggregation activity of the stored platelet is due to the reduction in the functioning receptor sides of GPIIb/IIIa on the platelet surface.

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QUANTITATION OF MICROPARTICLES IN PLATELET SUSPENSIONS BY FLOW CYTOMETRY.
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We have examined a platelet-poor, supernatant fraction from fresh and stored platelet suspensions with a FACS 440 (Becton-Dickinson) flow cytometer to study the distribution of small microparticles previously shown to be present in citrated plasma and serum (J. George et al., Blood 60: 834, 1982). Analysis by flow cytometry offers the advantage of discrimination of populations of particles by both light scattering and immunofluorescent properties. We found two distinctly different populations of particles: the predominant one had diameters in the range of 0.1 to 0.4um and was moderately autofluorescent (AF); the other was equally AF with particle diameters of 1.0 to 3.0um and probably included a few intact platelets. By adding a precise quantity of highly fluorescent beads of 0.9um diameter to each sample, relative concentrations of particles (small and/or large) could be quantified in platelet suspensions after various treatments using ratios of particle and bead counts. The lowest concentration of particles was found in samples from whole blood collected into CPDA-1 with PGE-1 and theophylline plus sodium azide (CPT-Az). Blood in CPDA-1 alone had twice the number of small and large particles; serum had a 20X higher particle concentration. A much larger number of particles was found in platelet concentrates (PC) stored for transfusion. Fresh PC had approx. 150X higher particle concentration than CPT-Az, rising to over 200X by the eighth day of storage at 22 C. Also, we noted a shift in distribution between particle populations in stored PC toward the larger size. The concentration of larger particles alone rose from 100X relative to CPT-Az to 350X after 8 days of storage. Similar changes in supernatant platelet factor 3 (PF3) activity were noted in stored PC in another study (A.P. Bode and D.T. Miller, Vox Sanguinis 51: 299, 1986), suggesting that supernatant PF3 activity may be related to one or the other population of particles seen by flow cytometry. This technique of examining and quantifying particles in platelet preparations by flow cytometry will facilitate and expand the characterization of platelet vesiculation and the released particles.