

## **Storage of Human Red Blood Cells and Platelets**

*Some aspects concerning the factors leading to storage lesion characterized as morphological changes and vesiculation.*

*Minireview based on a doctoral thesis*

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### **ABSTRACT**

1. Storage renders erythrocytes more responsive to thermally induced morphological changes, especially the shedding of microvesicles. 4-8 week old cells can be morphologically "rejuvenated" by heating.
2. If pH increases during storage of platelets an extensive loss of small particles occurs. The platelet disintegration is associated with a loss in the metabolic activity, discharge of LDH, increased susceptibility to phospholipid hydrolysis by phospholipase C and is found to be initiated during the actual preparation of platelet concentrates.
3. Activation of platelets during preparation can be decreased by shortening the first centrifugation time or by using adenine in the anticoagulant.
4. A 4 hour prestorage of the whole blood unit prior to centrifugation strongly decreases the activation of platelets upon stimuli and results in platelet concentrates much more stable to storage.

### **INTRODUCTION**

#### Biochemical and morphological changes in erythrocytes during storage.

Upon storage, a series of metabolic and morphological changes occur in the erythrocyte producing eventually a non-viable cell form that is rapidly removed from the body upon transfusion. To understand these changes, it is necessary to understand the structure and properties of the fresh erythrocyte.

The red cell membrane consists of a lipid bilayer of several different classes of lipids (30) with an asymmetric distribution of the individual lipid types (64). The choline-containing phospholipids (PC, SM) are located mainly in the outer layer and the amino phospholipids (PS, PE) preferentially in the inner layer.

Phospholipid composition in fresh human erythrocytes (30).

<u>Phospholipid class</u>	<u>Molar percentage</u>
SM Sphingomyelin	24.5±2.0
PC Phosphatidylcholine	25.4±1.1
PS Phosphatidylserine	5.8±1.7
PE Phosphatidylethanolamine	27.0±1.7
PIP <sub>2</sub> Phosphatidylinositol-4,5-bisphosphate	1.4±0.3
PI <sub>2</sub> Phosphatidyl inositol	1.2±0.1
PA Phosphatidic acid	2.2±0.2
Lysophosphatidyl choline	1.0±0.3
Lysophosphatidylethanolamine	1.3±0.3

Integral membrane proteins penetrate the lipid bilayer and interact with the hydrophobic lipid core. These include receptor and antigen-bearing proteins, such as glycophorin A and the anion transport channel Band 3. The latter is most important in that the protein cytoskeleton is anchored to Band 3. The red cell cytoskeleton consists of a highly, organized filamentous network mainly consisting of spectrin (band 1, 2), ankyrin (band 2.1), band 4.1 and actin (band 5) (13,49,50,78,79).

The metabolic changes occurring during storage result in membrane reorganisation and change in morphology. During storage the red cell undergoes a defined series of morphological changes (Fig.1)

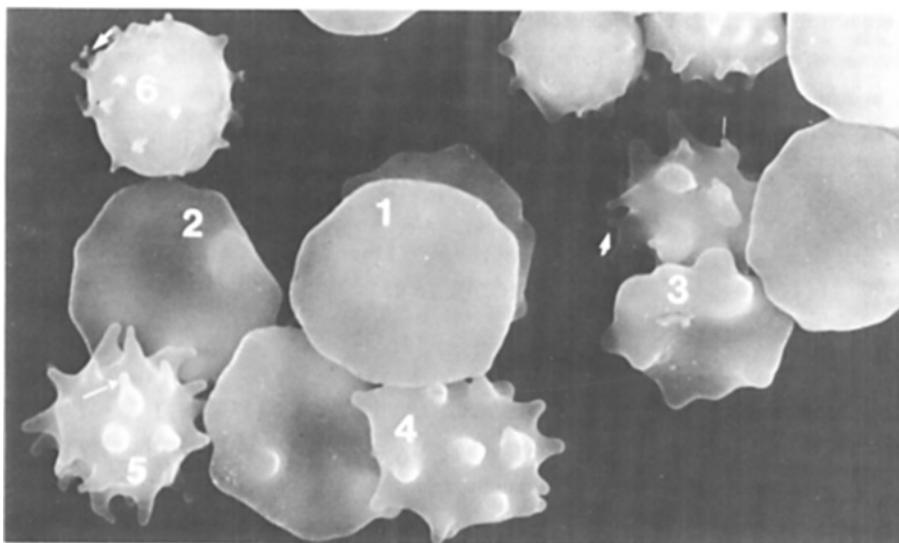


Fig.1. Scanning electron micrograph of stored red cells.

It passes from the smooth discocyte (1) to a crenated disc (2, echinocyte I; 3, echinocyte II) thereafter it swells to a crenated sphere (4, echinocyte III) and finally to a sphere with multiple short, thin spicules (5, sphero-echinocyte) that will gradually shed off (6, spherocyte) (16,45,47).

During storage intracellular  $\text{Ca}^{2+}$  increases and ATP decreases (25,33,37,73,96). The echinocyte formation and vesiculation (budding of the thin spicules) can be induced by treating fresh cells with

$\text{Ca}^{2+}$  and ionophore A 23187 (1,2). Echinocyte transformation can also be induced by metabolic depletion simply by incubating the red cells at 37°C for 24 h and results in two different reactions (69). One is the increase in lysolecithin in the outer phospholipid bilayer resulting in an expansion in the outer leaflet and echinocytosis (42), the other slower change is the suggested rearrangement of the membrane proteins (29).

ATP does not directly control either the form or deformability of the membrane, but events secondary to ATP depletion are responsible for the induced changes (28).

The degree of irreversible change upon metabolic depletion depends of many external factors such as: rate, length of time of ATP depletion, temperature, pH,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , plasma, oxygen availability. After about 30 h ATP depletion major lesions occur in the form of surface components loss (vesiculation) increase in cytoplasmic viscosity and osmotic fragility (93).

The earliest change in the membrane structure upon ATP depletion is the increase in the amount of spectrin-actin-4.1 complex that can be extracted from the membrane by exposure to low-ionic strength buffers (48). Band 4.1 is associated with the lipid membrane by a polyphospho-inositide ( $\text{PIP}_2$ , PIP)-regulated binding to glycophorin (5). The binding sites for actin and band 4.1 are localized to the tail of the spectrin tetramer. Near to the head region of spectrin is the binding site for ankyrin (89). The spectrin  $\beta$ -chain has four phosphorylation sites in the end of the molecule where the end-to-end association with the  $\alpha$ -chain occurs and close to the ankyrin binding sites. Ankyrin has also a phosphorylation site localized in the spectrin-binding domain (12). A conformationally intact cytoskeleton is essential for maintaining the cell shape and deformability. Upon heating a decrease in spectrin extractability can be measured prior to onset of red cell fragmentation and shape change (56).

Conformationally intact spectrin is necessary for preserving the membrane phospholipid asymmetry. When intact red cells are oxidised with agents that selectively crosslink spectrin, 30-50% of the inner membrane phospholipids, phosphatidylserine (PS) and phosphatidylethanolamine (PE), become accessible at the outer membrane surface (32). If these amino-phospholipids become exposed on the outer surface of the red cell or platelet, they will activate the conversion of prothrombin to thrombin and promote intravascular clotting (65,94). Exposure of PS and PE on the exterior of blood cells also makes them more adherent to endothelial cells (75) and increases the trapping into the reticuloendothelial cells (76). While the relationship between spectrin phosphorylation and shape change is only indirect (4,68), it is clearly evident that the phospholipid asymmetry and shape change are ATP-dependent (77). The maintenance of the membrane phospholipid asymmetry is due to two different processes:

- i) Binding of aminophospholipids to spectrin (32,80).
- ii) "Pumping" of lipids from the outside back to the inside, by the bidirectional ATP-dependent "flip-flop" enzyme (flippase) (92).

Although there is only 1.4% phosphatidylinositol-4,5-bisphosphate ( $\text{PIP}_2$ ) in the membrane it is evident that the conversion of  $\text{PIP}_2$  to PI is directly correlated to ATP depletion (30). Concomitant with this conversion phosphatidic acid will dephosphorylate to diacylglycerol. Taken together

the changes in lipid population will shrink the inner membrane monolayer by 0.6%, which is enough to convert discocytes to echinocytes III (10).

High levels of intracellular calcium activate the  $\text{Ca}^{2+}$ -dependent phospholipase C resulting in the degradation of PIP and  $\text{PIP}_2$  to the fusogenic diacylglycerol (3).

In conclusion, to survive in the circulation the erythrocyte must be both strong and flexible (49,50,57). The "cellular deformability" is necessary for passage through the microcapillaries and especially through the narrow portals of the spleen. The biconcave disc-shape of normal erythrocytes creates a surface area-to-volume relationship that is advantageous for this purpose. The sphere has in contrast to the discoid form minimum area-to-volume ratio and is therefore far less flexible and deformable. Stored cells not only lose in vivo viability because of increased trapping in the spleen but also disappear from the circulation because of increased adhesion to endothelial cells.

#### Preparation and storage of platelets

Whereas the human red cell is a rather simple non-nucleated cell consisting of a flexible and very stable, uniformly built up membrane enclosing a near-saturated solution of hemoglobin with the only function to transport  $\text{O}_2$  and  $\text{CO}_2$ , the platelet is a complex, non-nucleated, very active cell.

Platelets have, in addition, mitochondria performing oxidative phosphorylation and resulting in 36 ATP/glucose molecule instead of only 2 ATP/glucose in the glycolytic pathway. The adenine nucleotides in the platelets are enclosed in three physically distinct compartments, 64% in the dense granule pool (human ATP/ADP ratio = 0.7), 32% in the metabolic pool (ATP/ADP ratio = 4-5) and 4% in the F-actin bound pool (ADP only) (36).

The platelet plasma membrane is very similar to the erythrocyte membrane, the phospholipids are asymmetrically arranged in the same pattern (20,95).

Spectrin-like molecules underlying the membrane have been found (23) with concomitant changes in the organization of cytoskeletal elements and the exposure of internal phospholipids upon activation of platelets (9,21).

The platelet membrane has, in addition, several different receptors and is easily activated by many different agents (22,39,55). Many of these substances can also be released during the activation process itself such as ADP and serotonin (released from the platelet dense granulae), arachidonic acid and thromboxane  $\text{A}_2$  (from the phospholipid breakdown in the platelet membrane), thrombin (from the activation of prothrombin by the coagulation cascade) and collagen (released from injured epithelial cells). Activation can also be induced by physical stimuli such as cell-cell contact which occurs, for example, during mixing (6,7), centrifugation (53,74) and gel filtration (74). Activation of the platelet membrane results in the breakdown of phospholipids and especially in changes in the small phosphatidylinositol pool. The polyphosphatidylinositol ( $\text{PIP}_2$ ) hydrolysis to the second messengers inositoltriphosphate ( $\text{IP}_3$ ) and diacylglycerol (DG) is the initial agonist (e.g., thrombin, collagen, platelet activating factor, thromboxane  $\text{A}_2$ )-stimulated event in the phosphatidylinositol turnover (14,51,63).  $\text{IP}_3$  mobilizes  $\text{Ca}^{2+}$  from intracellular stores and initiates PI hydrolysis. DG

and  $\text{Ca}^{2+}$  in concert serve as a direct activator of protein kinase C resulting in the phosphorylation of the 40 kDa protein and the  $\text{Ca}^{2+}$ -enhanced serotonin release from dense granulae (39). DG, once produced, disappears very rapidly; it is converted either to phosphatidic acid or by the action of diglyceride lipase degraded to glycerol and arachidonic acid (11,39).

The  $\text{Ca}^{2+}$  mobilization also activates phospholipase  $\text{A}_2$  ( $\text{PLA}_2$ ), a stimulation that is further facilitated by DG (40) and results in arachidonic acid release from the major phospholipid pools (PC, PE, PS) (17,70). Liberated arachidonic acid is rapidly converted to prostaglandins and thromboxanes which in turn are capable of activating other phospholipids in the membrane resulting in a positive feedback loop (35).

The activation of PLC and  $\text{PLA}_2$  working together leads to an amplified response (8,17).

Both enzymes lead to arachidonic acid liberation even though they hydrolyse phospholipids in different ways (91).

PLC has an pH optimum of 7.5, is specific for phosphatidylinositol and liberates diglyceride.  $\text{PLA}_2$  activity is enhanced by increasing the pH (7.5 to 9.5), hydrolyses PC, PE, PS and liberates free fatty-acid and lysophospholipids (17). There is also a synergistic amplification in the platelet response as a result of simultaneously stimulation by different agonists (8,26,66).

In morphological terms the activation of platelets is seen as a change from a smooth disc to a sphere with pseudopods. The first phase in platelet activation is a reversible shape change. If the cell is more strongly activated a second phase will follow which is associated with release of the granulae content and irreversible changes occur (44).

A  $\text{Na}^+/\text{H}^+$  antiporter is involved in the pathway leading to arachidonic acid mobilization by epinephrine, ADP and low concentrations of thrombin (8). Removal of extraplatelet  $\text{Na}^+$  or increasing extraplatelet  $\text{H}^+$  (i.e. decreasing extraplatelet pH from 7.35 to 6.8) causes a blockage in the  $\text{Na}^+/\text{H}^+$  exchange system and inhibits platelet aggregation (88).

The morphological change in stored platelets is found to be well correlated with the posttransfusion viability and those concentrates having the highest proportion of discoid platelets also have the best survival (41). There is also a close correlation between the pH in the platelet concentrate (PC) after storage and the morphology (83). At pH 7.2-6.8 platelets maintain a normal discoid shape. With decreasing pH, platelets swell and at pH less than 6.0 all platelets are spherical (59). This fall in pH is due to lactate production from glycolysis (58). If the platelets are supplied with enough  $\text{O}_2$ , lactate production and thereby pH decrease is prevented. A new generation of storage bags with increased gas permeability has therefore been developed (61). The high gas permeability very effectively prevents the pH fall below 6.0. In the first generation of platelet storage bags the shelf life for PC was limited to 3 days because the rapid decrease in pH. In the second generation of platelet storage bags the shelf life has been extended to 5-7 days.

Loss of respiratory metabolism as observed by rising  $\text{pO}_2$  and decreasing  $\text{pCO}_2$  results in decreasing ATP levels. The platelet storage lesion, measured as the decrease in platelet count, increasing discharge of intracellular lactate dehydrogenase (LDH), decrease in the extent of photometrically measured shape change induced by ADP and the ability of platelets to recover from hypotonic shock are all found to be highly correlated to platelet ATP (34).

**For maintaining the phosphoinositide cycle, ATP is necessary. It has recently been shown that the**

generation of  $PIP_2$ , and not the second messengers  $IP_3$  and DG, controls the polymerization of actin and the mechanism of platelet shape change mechanism (43). The inability to produce  $PIP_2$  could therefore be a limiting factor for in vivo viability and function.

During storage the cells can start to bud off membrane (vesiculation) resulting in a irreversibly changed cell membrane. In the erythrocyte this is a relatively slow process and the uniformly built up cell membrane renders the erythrocyte a good model for studying the vesiculation process. In the platelet, the cell interior consists of several different organelles and an internal surface-connecting system (52). The main function of the platelet is the activation and aggregation process. The storage lesion of platelets in the form of budding/fragmentation, could, because of the risk for an accelerated "self-activation", be explosive. From an deeper understanding of the circumstances responsible for and the mechanism of vesiculation/fragmentation it will be possible to change the preparation and storage properties and in that way make more storage-stable, viable blood cells.

## RESULTS AND DISCUSSION

### A. Red cell storage and vesiculation

Fresh red cells are essentially unaffected by heating below the thermal transition temperature of spectrin ( $49^\circ\text{C}$ ), but above this temperature are very rapidly changed to large smooth spheres and subsequently to smaller smooth spheres (81).

During in vitro storage of red cells metabolic and morphological changes gradually occur. Some of the morphological changes are easily reversible, sometimes simply needing increases in intracellular ATP for reversibility, whereas some changes are irreversible e.g. the transformatin to spherocinocytes or sperocytes.

During storage of red cells to the time of outdated (4-8 weeks) the proportion of echinocytes(E)II and EIII is increased. A 1 minute heat treatment at  $46^\circ\text{C}$  of 4-8 weeks stored blood, readily increased the proportion of the discoid (D+EI) (i.e., morphologically "younger") cell forms (from 40 to 55%) concomittant with a decrease in the proportion of EIII. Recent findings of others also show that red cells, stored for 42 days in SAGM-solution, show a significantly increased (39% to 51%) percentage of discoid formed cells (D+EI) upon a one hour incubation at  $37^\circ\text{C}$ . This latter improvement in morphology does not, however, result in any change in the in vivo posttransfusion survival, potassium leak, glucose consumption or ATP concentration (38). It is thus highly questionable whether this morphological improvement we observe using the  $46^\circ\text{C}$  treatment represents a true "rejuvenation" of the aged erythrocytes.

If the stored cells instead are rejuvenated (by incubation with adenosine or adenine to raise the intracellular ATP level), the improvment in morphology is followed by an increased in vitro viability (37,47,90).

Prolonged heating of stored cells at  $46^\circ\text{C}$  resulted in a fall in the precentage discoid formed cells and a marked increase in the spherocinocytic cells (i.e., "accelerated ageing"). Thus indicating that there are still unrepaired changes in the stored cell membrane, making the cells more

susceptible to heating than are fresh cells.

At 48°C, extensive shedding of microvesicles and a gradual increase in the number of spherocytes occurs. At 50°C a rapid and extensive microvesiculation associated with a rapid conversion to spherocytes of different sizes is observed.

During ATP-depletion, spectrin will be dephosphorylated and result in echinocytosis. The loss of the interaction between the spectrin-cytoskeleton and the phospholipid-bilayer could explain the facilitated vesiculation occurring upon heating around the spectrin-transition temperature (67,78,81).

The spherocyte was found not to respond morphologically to heating other than by lysing.

Longster (47) has also shown, that stored cells which already have reached the "smooth" sphere state, were similar to the type of cells which were rapidly removed from the circulation upon transfusion. He also found that these cell types cannot revert to disc form upon incubation at 37°C with or without added adenine.

The spherocochinocyte, but not the earlier morphological forms, is also readily lysed upon treatment with phospholipase C (PLC, *Bacillus cereus*) and this enzyme can be used as a tool for assessing the degree of in vitro ageing of stored red cells (45). This would suggest some marked differences in the nature of the cell membrane in spherocochinocytes. Most probably a lowering of the lateral packing pressure in the membrane (24), although other membrane rearrangements cannot be excluded. Little (46) showed that glass-bottle stored RBC were more susceptible to PLC lysis than plastic bag (DEHP- containing) stored cells.

#### B. Storage of platelet concentrate in the first generation containers (PL-146).

The phospholipids of fresh human platelets are extremely resistant to hydrolysis by externally added PLC (82). This was also the case with activated fresh platelets. During storage, presumably as a result of decreases in the lateral packing of phospholipids in the external leaflet, the susceptibility to PLC degradation increases.

These suggested changes in membrane packing pressure occurring both in stored red cells and stored platelets could arise from a build up in the levels of components such as diglyceride, lysophospholipids or free fatty acids in the membrane (20).

The extent of phospholipid hydrolysis upon incubation of platelets with PLC is dependent on the plasma pH after storage (82). PC with a pH 6.6-6.8 resulted in only 10-15% phospholipid hydrolysis. Lower pH resulted in higher degradation. Increasing pH resulted in a more marked increase in the susceptibility to hydrolysis by PLC and this was found to be very extensive (50-60%) at pH>7.4. Note that the pH values refer to the plasma pH in the concentrates after storage. All platelets were incubated with PLC at the same pH.

In PC with pH>7.3 after storage, there was also a prominent change in morphology. The size distribution of PC shows that the platelets have become smaller and the PC contain an increased number of microvesicles/platelet fragments (83). A quarter of the platelets were balloon-formed or lysed and the discharge of LDH was elevated.

The storage lesion was also found to be more marked for PC of lower pH after storage. The progressive disc-to-sphere swelling was reversible as long as the pH was >6.1.

C. Storage of platelet concentrate in second generation containers (PL 1240, PL 732).

i) Influence of storage containers.

A limiting factor for platelet storage in the first generation of bags, was rate of diffusion of gases, especially O<sub>2</sub> through the bag walls. It was found that by using very thin-walled bags (60), other types of plastics (polyolefin, Fenwal type PL 732) (61) or by using other types of plasticizer in PVC bags (Fenwal 1240, Cuttler CLX) (31) the gas diffusion could be increased.

When PC stored in the first generation of bags (PL 146) were compared to PC in the second generation of bags (PL 1240), at storage expiry the latter were, in our hands, found on average to have become much more alkaline (84,85). In view of the well established connection between changes in pH during storage and loss of viability (41,58,60,61) the above observation would lead one to expect an enhanced loss of platelet quality in the second generation of bags. Indeed, this rise in pH during storage was associated with extensive evidence of platelet degradation in the form of decreased platelet count, high levels of extracellular LDH activity and very abnormal platelet size distribution profiles as shown in the Coulter Counter. This latter feature arose, from platelet disintegration as seen in electron microscopy (85) and with the subsequent appearance of large numbers of small particles (84). There was a strong correlation between the extent of pH increase during storage and the increase in relative number of small particles ( $r=0.85$ ). Isolation of these small particles showed that they *per se* had a very considerable procoagulant activity, measured as platelet factor 3 activity (PF3) (84). Similar findings have recently been found by Miller and Bode (54). Using a flow cytometer these workers have shown the occurrence of small particles in stored PC and that these particles have PF3 activity. They have also found that thrombin inhibitors in the anticoagulant inhibit thrombin production and the release of PF3 (18,19).

The discharge of LDH was also strongly correlated with the pH changes. In PL 1240 the LDH discharge was several-fold higher than that observed in PL 146 bags. The average LDH discharge in 3 PC after 4 days storage in PL 146 (i.e. storage life expiry) was 11% and in PL 1240 30% which rose to 45% after 6 days storage (storage life expiry for PL 1240 stored PC).

A major difference between PL 146 and PL 1240 is the nature of the plasticizer used in the manufacture of the plastic from which the storage bag is constructed.

In PL 146 DEHP (di-ethyl-hexyl-phthalate) plasticizer is used and this is easily extracted from the plastic, is fat-soluble and is known to be accumulated in the cell membrane (71). DEHP has been found to inhibit phospholipase A<sub>2</sub> activity and can thereby decrease the platelet aggregation ability (72). This chemical has also been shown to inhibit the vesiculation from erythrocytes (27). The storage life for red cell decreases from 35 to 21 days if types of storage bag, other than DEHP-containing ones, are used (62,71).

PL 1240 and CLX bags contain tri(2-ethylhexyl) trimellitate (TEHTM) as plasticizer. TEHTM is less fat soluble and results in a 30-fold reduction of plasticizer accumulated in platelet concentrates during 5 day storage at 22°C (i.e. 15 µg/ml of TEHTM compared with 450 µg/ml of DEHP) (31). The higher gas diffusion, in the second generation of bags, not only hinders the development of

anaerobic conditions and the resulting lactate production but concomitantly accelerates CO<sub>2</sub> diffusion out of the bags. An early rise in pH can sensitize the platelet to further damage resulting in less CO<sub>2</sub> production leading to further pH rise. A vicious circle resulting in high pH, loss of metabolic activity and platelet disintegration can in this way be established. Indeed, Murphy (61) has shown that deleterious changes and decrease in *in vivo* viability associated with high pH, could be largely prevented by storing PC in an atmosphere containing 10% CO<sub>2</sub>.

ii. Influence of preparation method.

Platelets are isolated from whole blood by centrifugation. By using different recommended centrifugation methods we found that the metabolic activity during and after storage in the PC was very different depending on the precise centrifugation method used (85,86). Already the day after preparation there was a highly significant difference between the pO<sub>2</sub> values in the bags (86). Depending on the platelet concentration, pH and the metabolic activity in the PC, the LDH discharge and the extent of build up of small paraticles (S.P.) after storage could be predicted, to 90% (LDH) and 86% (S.P.) respectively by the use of multivariate data analysis (86).

This close correlation between the aspects of the storage lesion and loss of metabolic activity was confirmed in the recently published results of Holme (34) showing the correlation between metabolic activity, ATP concentration and storage lesion.

Our findings concerning the strong influence of the centrifugation method used and the subsequent metabolic activity in stored PC was, however, in great contrast with experience reported widely in the U.S.A.

The major difference between the blood collection and handling procedures in U.S.A. and our own is the time between blood withdrawal and subsequent preparation. In the U.S.A. about 80% of the blood is collected in blood-mobiles, resulting in a gap of several hours between donation and the separation of blood components. In Sweden and Norway the preparation of components is supposed to be carried out within two hours after phlebotomy (15).

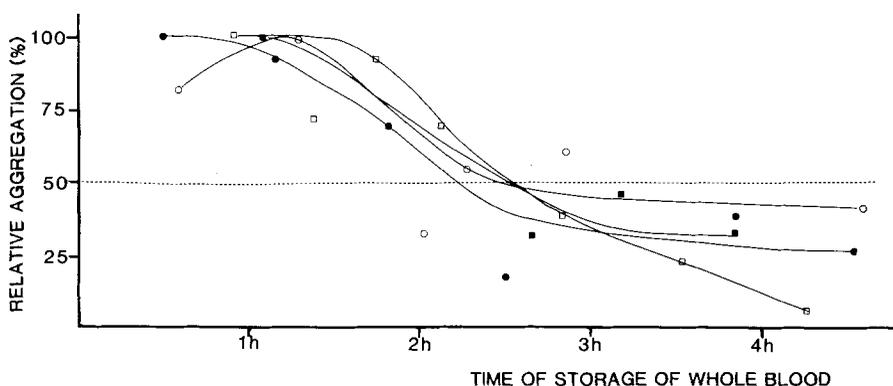


Fig. 2. Changes in aggregation response in whole blood upon storage from four different donators.

With the use of a whole-blood agglomerator, changes in the aggregation response after phlebotomy could be followed in whole blood, i.e., without any manipulations of the blood (Fig. 2). The aggregation response measured at 37°C starts to decrease after 2 hours. After 3 hour the aggregation was less than half the maximum response (87). Preparation of PRP after a 4 hour prehold of whole blood results in a significantly decreased aggregation response upon stimulation with collagen. Using lower (cyclooxygenase-dependent) doses resulted in no aggregation (measured as change in light absorption), whereas PRP prepared exactly 60 minutes after blood withdrawal yielded a high aggregation response.

Using CPD-Adenine instead of CPD alone as anticoagulant was also found to decrease significantly the aggregation response to physiological doses of collagen.

The superior morphological state of 6 day-stored platelet concentrates from platelets prepared after 4h-rested blood compared with immediately processed blood is clearly apparent in electron microscopy (Fig.3.)

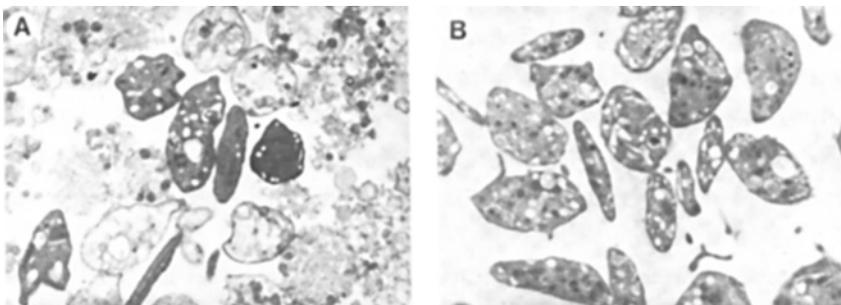


Fig.3. Transmission electron micrographs of 6 day-stored PC. A= PC prepared close to phlebotomy showing a high number of lysed or fragmented platelets, which correspond to the high LDH discharge. B= PC prepared after 4 h prestorage of whole blood showing discoid platelets with well preserved internal structure and almost no lysed cells.

**A 4 hours prehold of CPD-whole blood prior to preparation in the second generation of bags results in less activation during preparation and less storage lesion.**

The combination of storage at higher pH and no DEHP in CPD-plasma renders the time delay before preparation as a new important parameter to be considered in platelet storage.

In Norway and Sweden and conceivably in other regions of the world as well, present blood bank practise is to process blood as soon as possible after donation. In such a situation, the combination of the second generation storage containers and adenine-free anticoagulant can very easily yield substandard, potentially dangerous platelet preparations after storage. The uncovering of this fact together with what appears to be a very simple way of avoiding this problem is undoubtedly the most important aspect of the work described in this thesis.

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