Doc. 357/31

THE APPLICATION OF PLASTICISED POLY(VINYL CHLORIDE) MATERIALS FOR THE COLLECTION, STORAGE AND DELIVERY OF BLOOD AND BLOOD COMPONENTS : A CRITICAL REVIEW.

COLIN R. BLASS CChem MRSC

This thesis is submitted in partial fulfilment of the degree of Master of Philosophy from the Bioengineering Unit of the University of Strathclyde.

August 1992

Bioengineering Unit,
University of Strathclyde,
Wolfson Centre,
Glasgow, G4 0NW.

The copyright of this thesis belongs to the author under the terms of the United Kingdom copyright acts as qualified by University of Strathclyde Regulation 3.49. Due acknowledgement must always be made of the use of any material contained in, or derived from, this thesis.'

This is to declare that this project is entirely my own work and has not previously been submitted to this or any other University.

Signature

Colin R. Blass

ABSTRACT

Plasticised poly(vinyl chloride) is one of the most widely used biomaterials. A critical review has been undertaken of plasticised poly(vinyl chloride) in relation to the collection, storage and delivery of blood and blood components. Following consideration of the classification of whole blood and blood products, an examination is made of the blood response, the composition of plasticised poly(vinyl chloride) polymers and relevant regulatory requirements. A focus of interest in poly(vinyl chloride) biomaterials is plasticiser selection and this is reviewed in terms of the toxicity of polymer formulations, with an emphasis on di (2-ethyl hexyl) phthalate, the principal plasticiser employed. Di (2-ethyl hexyl) phthalate, is considered further with respect to the distribution of the plasticiser and its metabolites in blood and blood components and the influence of plasticised poly(vinyl chloride) on the survival of stored red blood cells. The review is completed by a discussion of containers for blood platelets and the possible development of biomaterials based on novel plasticisers. The review balances criticism of plasticised poly (vinyl chloride) by a presentation of beneficial processing characteristics and relevant properties.

CONTENTS

ACKNOWLEDGEMENTS

I would like to express my thanks to both Professor J.P. Paul and Professor J.C. Barbernel for their guidance and support during this project.

To my supervisor Professor J.M. Courtney, I wish to express my sincere gratitude for his advice, enthusiasm and encouragement which made my period of study such an enjoyable experience.

I am grateful to the members of staff and post graduate students within the Bioengineering Unit who provided valuable advice and assistance when as an external student I was present on campus.

My thanks to Hydro Polymers who provided financial support, the secretarial services of Rona Morley at Newton Aycliffe, Pip Edgar and her hard working team at PEP, and Dr M.S. Biggs who was frequently consulted in matters concerned with regulatory affairs.

Finally a special thank you to my wife Jill and son Timothy for their patience, encouragement and interest in this thesis.

ABBREVIATIONS

ATBC	acetyltributyl citrate
ACD	acid citrate dextrose
ADP	adenosine diphosphate
ATHC	acetyltri-n-hexyl citrate
ATP	adenosine triphosphate
ATIII	antithrombin III
AIDS	acquired immune deficiency syndrome
BTG	beta-thromboglobulin
BHBB	1,3,5-trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl) benzene
BTHC	n-butyryltri-n-hexyl citrate
CEFIC	European Council of Chemical Manufacturers' Federation, Brussels
CPD	citrate phosphate dextrose
CPDA-1	citrate phosphate dextrose adenine
DEHP	di(2-ethylhexyl)phthalate
DMSO	dimethyl sulphoxide
2,3 DPG	2,3 diphosphoglycerate
EDTA	ethylenediamine tetraceticacid
EEA	poly(ethylene-co-ethyl acrylate)
FDA	Federal Drug Administration - United States of America
G-6-PD	glucose-6-phosphate dehydrogenase
HES	hydroxyethyl starch
HIV	human immune deficiency virus
HSR	hypotonic shock reaction
MEHP	mono(2-ethylhexyl)phthalate
NSA	normal serum albumin
PC	platelet concentrates
PPF	plasma protein fraction
PRP	platelet rich plasma
PA	polymeric adipate
PO	polyolefin
PVC	poly(vinyl chloride)
PVP	polyvinylpyrrolidone
RBC	red blood cells
SR	silicone rubber
TEHM	tri(2-ethylhexyl)trimellitate
TET	tri(2-ethylhexyl)-1,2,4-benzene tricarboxylate
TOTM	trioctyl trimellitate
VCM	vinyl chloride monomer

CHAPTER 1

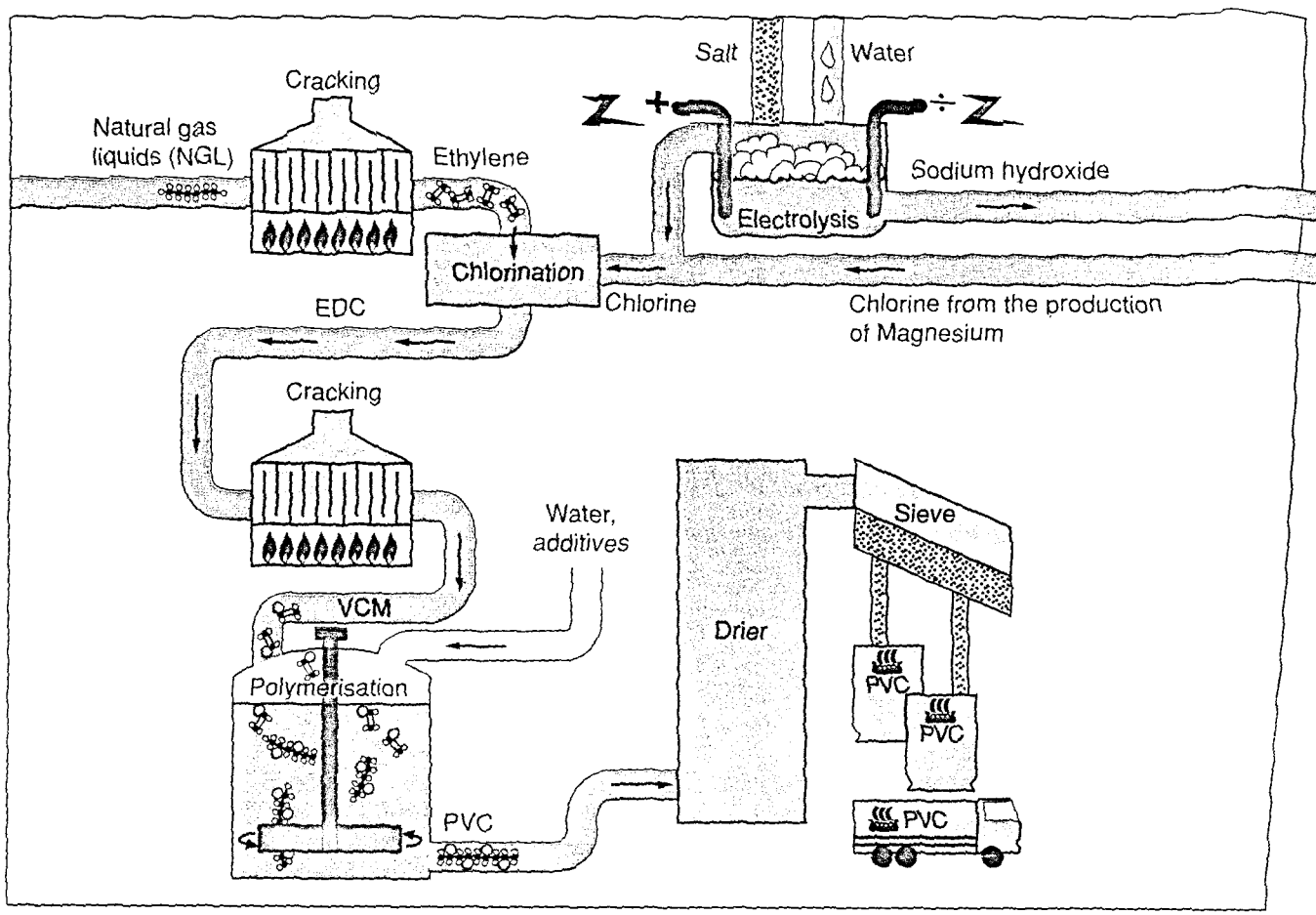


Fig 1.1 **PVC Polymer Production**

CHAPTER 1.
INTRODUCTION

1.1 POLYMERIC BIOMATERIALS

The compatibility of polymeric materials with the human body, or animals during screening, is an important factor in their biomedical use. In most cases, only relatively benign polymers can be tolerated for medical usage, but there are certain applications where a specific interaction between the polymer and the body's tissues may be desirable and necessary for successful operation of the material or device. This precludes an all-encompassing definition, although a generally accepted definition is the "no definition", Klinkmann et al, (1984, 1987), which implies that a biocompatible material must not induce any thrombogenic, toxic, allergic or inflammatory reactions and must cause no deformation of cellular elements, no change in plasma proteins or enzymes, no immunological reaction, no carcinogenic effect and no deterioration of adjacent tissue. Clearly, polymers fail to meet these criteria and since the body is composed of a large variety of distinctly different kinds of tissues, it can readily be envisaged that a polymer might be biocompatible in one bioenvironment and incompatible in another. Ultimately, the crucial test of a biomedical material or device occurs when it is in contact either directly or indirectly with the patient. Good medical practice requires prior knowledge that the material or device will not present undue risk to the patient, and will fulfil the task for which it is intended. In the context of this thesis, the term haemocompatibility may be considered to be more appropriate as it relates specifically to blood-material interactions.

1.2 POLY(VINYL CHLORIDE) BIOMATERIALS

The raw materials used in the manufacture of poly(vinyl chloride) or PVC are derived from sodium chloride (57 percent) and oil or gas (43 percent). The relatively modest oil content in PVC reflects the fact that its production requires a smaller share of the world's limited oil reserves than almost all other bulk thermoplastics. There is, of course, an almost unlimited supply of sodium chloride in the form of common salt.

The starting point for a typical PVC production process operated as shown in fig 1.1 commences with natural gas liquids extracted from the North Sea. The ethylene produced from the cracking process is reacted with chlorine to form the intermediate ethylene dichloride (EDC), which is catalytically cracked to form vinyl chloride, the monomer for PVC production. Polymerisation takes place either in suspension, to produce S-PVC, or as an emulsion to provide the basis for plastisol and paste making PVC polymers. A number of S-PVC's are produced, which display a range of physical property parameters related to their molecular weight distribution and morphology. The higher molecular weight types provide optimum mechanical strength

Rigid Mouldings

- Check valves
- Connectors
- Needle Hubs

Flexible Containers

- Blood and blood components
- Enema packs
- Intravenous solutions
- Nutritional fluids
- Urine drainage

Flexible Extrusions

- Blood taking/giving sets
- Cannulae
- Catheters
- Endotracheal tubes
- Guedel airways
- Haemodialysis sets
- Heart-lung bypass sets
- Intravenous Solution giving sets
- X ray opaque tracer filaments

Fig 1.2 PVC Medical Device Applications

characteristics combined with porous polymer morphologies, enabling rapid absorption of selected plasticisers and are therefore chosen as the basis for compounds destined for the manufacture of flexible containers and tubing for blood collection, storage and administration.

PVC polymers, while almost inert in their purest form, and therefore closest to any biocompatibility definition, poses both thermal instability and brittleness, which prevent them from being directly processed and fabricated into components for medical use. It is the ability of PVC polymers to accept a wide and diverse range of chemical additives that enables the resulting PVC compounds to find such a wide scope in medical device applications (fig 1.2).

In the simplest cases, rigid PVC formulations for needle hubs, drip chambers, connectors and check valves would be based on a high purity medical grade of S-PVC incorporating a methacrylate butadiene styrene (MBS) impact modifier, acrylic processing aid, organo-tin or calcium/zinc-based heat stabilising system, lubricants and perhaps a tinting agent or colourant. The S-PVC would always be the major component and normally represents 90 to 95 per cent of the final composition.

Where flexible PVC is concerned, the level of selected plasticisers present often equals or exceeds that of the base polymer depending on the degree of flexibility, and modifiers and processing aids may be absent. The flexibility of PVC materials, often measured by quoting a Shore A or British Standard Softness Number (BSS), may be adjusted to suit any particular requirement by the incorporation of a known concentration of a specified plasticiser. Plasticisers depending on their molecular weight or structure display differing plasticiser efficiencies, which should be borne in mind when considering plasticiser extraction into biofluids. As a result of its relatively low cost and high plasticising efficiency di(2-ethyl hexyl) phthalate has remained the single most important plasticiser in the majority of medical devices incorporating flexible PVC components.

The present annual demand for PVC in Western Europe is approximately 5.1 million tonnes. In 1990, world capacities amounted to more than 20 million tonnes. Currently, some 45,000 tonnes of PVC are consumed in the manufacture of medical devices in Western Europe and this consumption amounts to slightly less than one percent of total demand. This figure represents PVC used directly or in close contact with patients and not the higher figures often quoted which include items of hospital equipment such as outer packaging for medical devices and oxygen tents. The use of the major polymers in medical applications in Western Europe is illustrated in fig 1.3. In terms of volume, PVC remains the most widely used polymeric biomaterial for single use, pre-sterilised medical devices.

Historically, PVC was introduced into flexible containers and tubing some 40 years ago as a replacement for glass and natural rubber and began to dominate the market when the need for single use, pre-sterilised components emerged. The major factors that continue to favour its use are

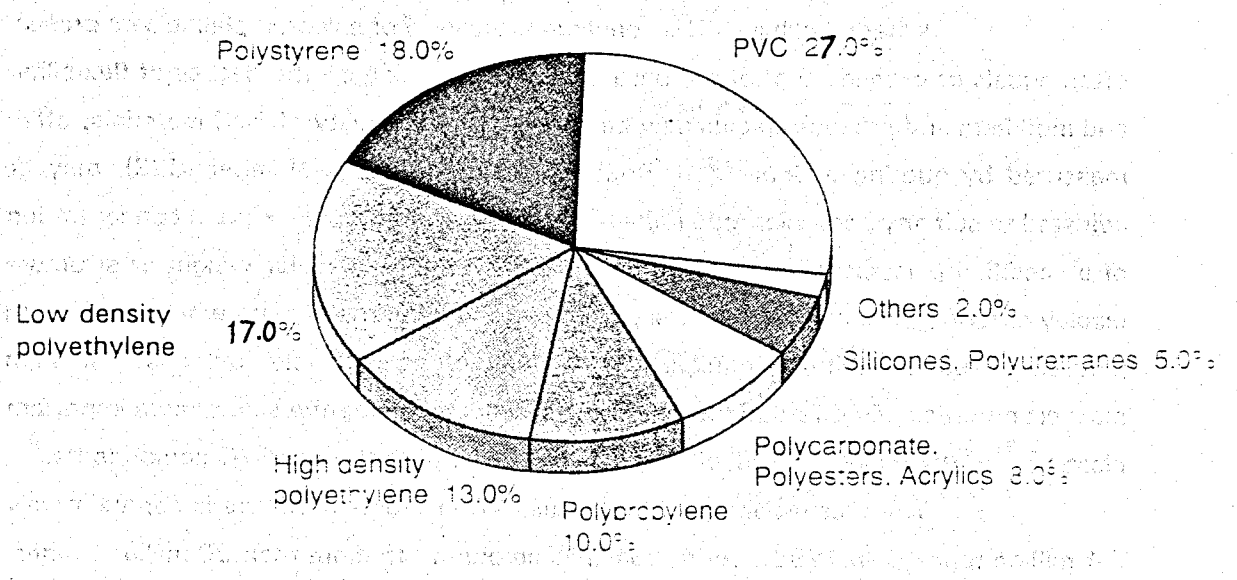


Fig 1.3 **Use of plastics in medical applications in Western Europe (1990), (Blass, 1992)**

- low toxicity
- chemical stability
- high frequency weldability
- sterilisation performance
- cost effectiveness

The additional specific attributes which flexible PVC displays and are necessary in the design and function of blood-bags are ease of fabrication in converting the appropriate compounds by extrusion, moulding or calendering processes to the complex array of multi components which go to make up a typical blood pack unit.

1.3 BLOOD COMPATIBILITY

When blood contacts an artificial surface it initiates a complex series of reactions (Szycher, 1983). It is generally believed that plasma protein adsorption is the first event which occurs and that subsequent phenomena are to a large extent determined by interactions of the blood with the adsorbed protein layer. The blood response to the PVC surface during storage is complicated by the tendency of the artificial surface to induce thrombus formation and the consequent need to introduce an antithrombotic agent to enable its effective clinical application. The features of the blood response influenced by the biomaterials can be classified under 6 headings.

(i) Protein adsorption
(ii) Platelet reactions
(iii) Intrinsic coagulation and contact activation
(iv) Fibrinolysis
(v) Leucocyte alterations
(vi) Complement activation

(Courtney et al, 1989)

Each one of these features covers a multiparameter, complex series of reactions which are interrelated. While the monitoring of the blood response in procedures such as haemodialysis and cardiopulmonary bypass is of great importance as it can be expected with a greater understanding to lead to improvements in blood compatible materials, its relevance in the application of flexible PVC materials for blood storage is outweighed by other factors such as plasticiser extraction and gas permeability properties. It is however interesting to note that the hydrophobic nature of the PVC surface displays a greater affinity for the plasma proteins (Chuang et al, 1978) than for a Cuprophan dialysis membrane which represents a hydrophilic surface.

membrane which represents a hydrophilic surface.

1.4 THESIS OBJECTIVE

The aim of this project was to present, with a single critical review, features of flexible PVC polymers relevant to the collection, storage and delivery of blood products. It was intended that such a review would offset unjustified criticism of PVC by a clear presentation of beneficial properties and promote the following:

1. Assistance to non specialists in reaching conclusions with respect to PVC.
2. Research to improve the performance of PVC biomaterials.

CHAPTER 2

CHAPTER 2.

CLASSIFICATION AND APPLICATION OF WHOLE BLOOD AND BLOOD PRODUCTS PACKAGED IN POLY(VINYL CHLORIDE) CONTAINERS

2.1 INTRODUCTION

The British Pharmacopoeia Volume II (1988) contains a section entitled "Blood Preparations", which includes whole human blood and various blood components and plasma fractions. The term whole blood refers to anticoagulated blood from which none of the components has been separated. A blood component is that portion of blood which is separable by either physical or mechanical means, such as differential centrifugation while a plasma fraction is a derivative of plasma obtained by chemical processes, such as alcohol precipitation. In modern blood transfusion practice, blood component therapy is advocated and this refers to the selective transfusion of blood components or plasma fractions, in preference to the routine use of whole blood. The term blood products is normally applied to blood components and plasma fractions but is sometimes confined to plasma fractions only.

The introduction of a system of flexible poly(vinyl chloride) packs with integral tubing for collection, processing and storage, together with the concurrent development of high speed refrigerated centrifugation have provided the means of preparing sterile blood components. Similarly, protein fractionation centres with adequate fraction capacities and using sophisticated chemical separation techniques provide concentrated plasma fractions instead of whole blood.

It is now possible to select the blood products which are most likely to correct the specific deficiencies in the blood of patients. The appropriate blood product can be transfused in a concentrated form, thus correcting the deficiency and achieving effective blood levels in the recipient, with the administration of a small volume and without causing circulatory overload. Specific component therapy conserves a valuable human resource and a single donation of whole blood may be used to treat several patients (Wallace, 1977).

2.2 BLOOD DONATION

The National Blood Transfusion Service in the United Kingdom, in common with many transfusion services, bleeds donors not more than twice a year and does not bleed women who are pregnant or who have been pregnant within the previous year. The major objective of this policy is to protect the donor from iron deficiency. A number of other conditions which may disqualify a donor include :

(i) persons carrying transmissible diseases such as viral hepatitis in its several forms, Acquired Immune Deficiency Syndrome (AIDS) and other syndromes caused by the Human Immune - deficiency Virus (HIV), malaria, Changas' disease, brucellosis and syphilis.

(ii) allergic subjects, as they may passively transfer their sensitivity to the recipient for a short period.

(iii) patients having received recent inoculations or vaccinations, to avoid the possibility of transmitting a live virus (measles, yellow fever, rabies, vaccinia and rubella) or killed vaccines (hepatitis B, typhoid, influenza, cholera) or toxoids.

(iv) patients having undergone blood transfusions and tissue grafts within six months, or surgery without blood transfusion within one to three months depending on the underlying condition.

(v) individuals under medication due to their clinical condition or those addicted to drugs injected by the parenteral route.

(vi) persons with a specific medical condition or trait, e.g. coagulation factor deficiencies and relatively minor red cell abnormalities, such as sickle cell trait and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency.

As a general rule in the United Kingdom, any adult aged between 18 and 65 who is in good health and has not recently had any serious illness, is a suitable donor. Careful records are kept of all donors with information on blood groups, evidence of alloimmunization, presence of microbial antibodies and frequency of donation. The volume of blood donated varies from country to country but in the United Kingdom 450ml (± 45ml of blood together with samples of 20-30 ml are taken provided donors weigh at least 50kg, or 250ml in cases where the weight of the donor falls between 43.6 and 50kg.

2.3 WHOLE BLOOD

Whole blood is the starting material from which all blood products are derived and is described in detail in the British Pharmacopoeia (Volume II 1988). One of the prerequisites to the collection and storage of donor blood is the addition of a substance which prevents coagulation and the British Pharmacopoeia simply states that whole blood is blood which has been mixed with a suitable anticoagulant.

2.3.1 Anticoagulants

One example is given in the above text, namely a sterile pyrogen free citrate solution of acid reaction containing dextrose: when using 120ml of the particular acid citrate dextrose (ACD) for the collection of 450ml of donor blood, it is stated that the red corpuscles of the blood may be preserved for 21 days at a temperature of 4° to 6°C. Anticoagulants should not cause deterioration of the red cells and must be non-toxic to the recipients: preservations such as glucose may be added to inhibit the deterioration of the erythrocytes. Acid citrate dextrose (ACD) contains sufficient citrate to bind the free calcium ions in donor plasma and thus prevent coagulation. A storage temperature of 2° to 6° has an inhibiting effect on red cell enzymes controlling glycolytic metabolism and the added preservative helps

to maintain the concentration of glucose at a level which gives adequate viability of the red cells for a storage period of at least 21 days. Conventionally, the acceptable shelf life of red cells stored at 2° to 6°C is determined by demonstrating that at least 70 per cent of the red cells stored for a certain period remain in the circulation 24 hours after transfusion. Although there is a wide range of in vivo survival values, the mean survivals of red cells indicate in terms of this conventional survival, that ACD blood may be stored for at least 21 days. Transfused red cells which survive for 24 hours will then disappear from the circulation at a normal rate. In the United States, whole blood is banked at 2° to 6°C for up to 49 days. The safety of citrate for i.v. administration was first described by Hustin (1914), who probably gave the first transfusion of citrated blood to a human. Citrate is the most widely used anticoagulant in whole blood storage. Its advantage is that is prevents coagulation over a long period in stored blood.

The sodium salts of ethylene diamine tetra-acetic acid (EDTA) are powerful chelating agents and also prevent coagulation by binding calcium ions. Although the binding of EDTA with free calcium ions is more powerful than that of citrate, it is no longer used in blood transfusion work because in practice it offers no advantage over citrate and also gives rise to platelet damage.

Heparinized blood is rarely used at present and heparin is not recommended for routine blood collection as it is not a preservative solution and its anticoagulant properties are neutralised by normal plasma. Heparinized blood should be transfused within 24 hours of collection. An indication of its low toxicity is given by the fact that a subject may be heparinized, i.e. given a sufficient dose of heparin to render his blood temporarily incoagulatable with very little risk and is therefore a routine practice in many open-heart surgery units. Heparin prevents coagulation by inactivating the proteolytic activity of thrombin, after complexing with antithrombin III (ATIII) and thrombin (Barrowcliffe et al, 1978). By increasing the activity of ATIII, which is a serine-protease inhibitor, heparin also inactivates factors Xa, IXa and plasmin (Wessler & Gitel 1979). Heparin is not a suitable anticoagulant for plasma fractionation, since it reduces the yield of factor VIII by allowing the formation of fibrinogen in stored plasma.

2.3.2 Whole Blood Storage

The British Pharmacopoeia Volume II (1988) lays considerable emphasis on microbiological aspects, while no mention is made in the monograph of various changes which occur during storage of whole blood, affecting particularly formed elements, protein fractions and biochemical properties. Platelets survive poorly and it is unlikely that a donation stored for more than 24 hours will provide functionally active platelets. Platelet preparations used to treat thrombocytopenia are derived from fresh whole blood, fresh platelet-rich plasma or platelet concentrates. Similarly, leucocytes show rapid functional deterioration, but in any event the number of granulocytes in a single donation of fresh whole blood is so small that

other means of providing granulocyte therapy must be used.

The most important storage changes in the plasma of whole blood kept at 2° to 6°C are a loss of labile coagulation factors V and VIII, together with altered biochemistry including an increase in potassium content. Fresh plasma separated from whole blood, preferably within four hours of the donation, should be used as the starting material for Factor VIII products. The diffusion of potassium from red cells during storage of whole blood causes the plasma potassium to increase at a rate of approximately one m mol^{-1} per day. The average content of plasma potassium in whole blood stored for 21 days is 23 m mol^{-1}. There is also an increase in the concentration of plasma ammonium pyruvate and lactate ions during storage of whole blood and a decrease in the pH of plasma. The plasma pH, measured at 37°C, of fresh ACD blood is 7.0, whereas after normal storage as whole blood for 21 days it is 6.7. These considerations together with the presence of particulate matter are recognised when whole human blood is used in massive replacement therapy in the exsanguinated patient and there may also be a need to provide supportive component therapy.

The collection and storage of whole blood present a dilemma in patient care. One, the desire to provide donor blood in which the components function in the same way in the recipient as they did in the donor and two, the ready availability at all times of adequate quantities of donor blood to meet urgent clinical requirements. The transfusion of whole blood provides for those emergency clinical situations where it is beneficial to supply the patient simultaneously with oxygen carrying red cells and plasma volume expanding proteins. Until recently, a compromise had been accepted in that storage of whole blood for three weeks at 4°C had ensured a ready availability, but meant a reduction in quality compared with fresh blood. The modern practice of blood component therapy aims to provide adequate amounts of high quality and safe blood products.

Donor blood should be promptly cooled to below 15°C after collection to prevent the fall in 2,3 diphosphoglycerate (2,3 DPG) levels which promotes oxygen transfer from red cells.

Poly (vinyl chloride) plasticised with di(2-ethyl hexyl) phthalate is currently the most widely used packaging medium for the storage of whole blood. Its influence on the application of clinical haematology is the subject of subsequent chapters.

2.4 RED BLOOD CELLS

The British Pharmacopoeia Volume II (1988) describes a blood preparation entitled concentrated human red corpuscles, which is prepared from whole human blood by the removal of a quantity of plasma and anticoagulant solution, equivalent to not less than 40 per cent of the total volume of the whole human blood. This preparation is also referred to as packed red cells because the donation of whole blood is centrifuged to pack the red cells before removing the supernatant citrated plasma. The amount of citrated plasma removed results in red cell concentrates with a haemocrit value of 70-80 per cent (e.g. mean

corpuscular volume x red cell count). These packed red cell concentrates have been used to treat patients with severe anaemia where the volume added by transfusion may precipitate circulatory overloading and cardiac failure. The risk of bacterial contamination must be avoided in the preparation of red cell concentrates when separating the supernatant plasma from the cells. This risk has been greatly reduced with the replacement of glass bottles by plastics and red cell concentrates are stored for the same period as whole blood.

Clinical requirements and technical advances have resulted in the provision of a number of red cell preparations of the following types :

(i) concentrated or packed red cells with or without the buffy layer

(ii) partially packed red cells

(iii) washed red cells

(iv) frozen red cells

(v) leucocyte-poor and platelet-poor red cells.

These various forms of red cell preparations are inter-related to meet the transfusion therapy of individual patients and support the general application of component therapy. The red cell preparations may be stored under the same conditions as whole blood apart from frozen red cells which provide a special case.

2.4.1 Frozen Red Cells

Satisfactory storage of red cells in the frozen state became possible when it was discovered that these cells when mixed with glycerol could be frozen and thawed without damage (Smith 1950). The unlikely way in which the protective effect of glycerol was discovered has been described by Sloviter (1976). The method of preserving red cells in the frozen state and recovering these red cells for transfusion therapy results in the removal of most of the leucocytes, platelets and soluble protein antigens, which have been present in the original donation of whole blood. Detailed consideration of the preservation of red cells at low temperatures has been given (Blagdon 1972; Mollison 1972).

Mechanical refrigeration at minus 80°C is used for the preservation of red cells in the presence of high glycerol concentrations (40-45 per cent final w/v), which provides a shelf life of around two years.

The use of cryoprotective agents decreases haemolysis during the freezing and thawing of red cells, which would occur as a result of the concentration effect into solute as the solvent water crystallizes out of solution. The cryoprotective agents used for the long term preservation of human red cells of transfusion therapy are of two types, extracellular and endocellular. Examples of extracellular agents are polyvinylpyrrolidone (PVP) and hydroxyethyl starch (HES). Endocellular agents, of which glycerol is an example, include dimethylsulphoxide (DMSO), dextrose, lactose and sucrose. In the presence of appropriate endocellular or extracellular cryophylactic agents, the preservation of red cells by the so called low glycerol concentration method (15-20 per cent final w/v) is performed in liquid nitrogen

at minus 196°C, with subsequent storage in the gas phase at -160°C. The shelf life of the product from the low glycerol method is still clinically acceptable after 10 years cryopreservation. The standard plasticised poly(vinyl chloride) packs used for blood storage at 4°C disintegrate in liquid nitrogen and the glycerolized red cells are preserved in aluminium storage canisters.

2.5 BLOOD COMPONENTS

Blood components arising from the high speed refrigerated centrifugation provide a number of blood components in addition to red cells, namely platelets, granulocytes, whole plasma and cryoprecipitate (factor VIII).

2.5.1 Platelets

In modern blood component therapy, flexible polymeric containers are now used for the collection of platelets, firstly, because their use is almost essential in the preparation of platelet concentrates and secondly, because their use is absolutely essential if the concentrates are to be stored, since the platelets are separated in a closed system and gas exchange through an appropriate polymer is vital to avoid hypoxic conditions leading to an accelerated production of lactic acid. The availability of polymeric materials with greater gas permeability characteristics or thinner wall constructions has prolonged the shelf life of platelet concentrates through enhanced gas exchange mechanisms (Rzad et al, 1982; Gunson et al, 1983; Simon et al, 1983; Koerner, 1984; Murphy et al, 1984; Rock et al, 1984a; Holme et al, 1989).

Platelet survival is poor in stored whole blood and all platelet preparations must be obtained from individual donations of fresh whole blood. It is preferable therefore that the starting material should be absolutely fresh and donations of whole blood which have been stored for more than 24 hours provide relatively few functioning platelets for successful treatment of thrombocytopenia. The anticoagulant commonly employed is ACD, but fresh citrate phosphate dextrose (CPD) whole blood yields satisfactory platelet preparations.

Greenwalt and Perry (1969) have discussed variations in the preparation of platelets, including centrifugal force, temperature of preparation, acidification of platelets, agitation of platelet concentrates and the temperature and duration of storage. A unit of whole blood is collected into the primary pack of a multiple system of polymeric packs. This fresh donation in the primary pack is then centrifuged slowly and the supernatant platelet rich plasma (PRP) is expressed through the connecting integral plastic tube from the primary pack to a satellite pack. The primary pack can be disconnected from the satellite pack, leaving a red cell suspension or concentrate in the primary pack. The platelet-rich plasma in the satellite pack may be used as PRP or processed further to produce platelet concentrates (PC) or other products. To prepare PC, the PRP is centrifuged rapidly, leaving the platelet-poor supernatant plasma, which can be removed to provide other components and fractions. Approximately

20ml of plasma are left on the sedimented platelets in order to resuspend the platelet concentrate and facilitate their administration to the patient.

Platelets are usually stored as concentrates and resuspended in autologous plasma or in a plasma-free medium, such as modified Tyrode's Solution (Rock et al, 1985). After storage for 5 days, recovery and survival are the same as those of platelets stored for the same time in plasma (Adams et al, 1986).

Platelets are stored under several temperature conditions which offer both advantages and disadvantages as the following indicates. Platelets stored at 22°C under conditions currently considered to be optimal, exhibit profound functional abnormalities in conventional in vitro tests of aggregation and release. However, these abnormalities are reversible according to Murphy and Gardner (1971) and platelet post transfusion survival is good. Platelets stored at 4°C lose their discoid shape and their viability and, although their immediate post transfusion recovery is good and they retain some haemostatic function, their subsequent survival is poor. Storage of platelets is also undertaken in the frozen state at -80°C.

Autologous platelets stored in CPD at 22±2°C for 72 hours were found to have a mean recovery of 46% and a mean survival of 7.9 days. Storage in ACD gave similar results (Slichter and Harker, 1976). No differences in recovery or survival of platelets preserved in CPD or CPDA-2, even after 8 hours storage of whole blood at room temperature, were found by Bolin et al (1982). In thrombocytopenic recipients, platelets stored for 72 hours had a recovery of 42% and a mean survival of 4.1 days (Slickter and Harker, 1976). Storage in CPDA-1 gave very similar results, i.e. for autologous platelets stored for 72 hours recovery was 50% and survival 7.3 days; in thrombocytopenic subjects, recovery averaged 44% and survival 3.3 days (Scott and Slichter, 1980). A major limiting factor in storage at 22°C is the fall in pH due to lactate production from platelet glycolysis (Murphy et al, 1970). When pH falls to 6.8 platelet morphology begins to change and it changes dramatically when pH reaches 6.0 and viability is lost. The rate of fall of pH is affected by the number of platelets, the volume of plasma in which they are stored and the availability of oxygen, since deprivation of oxygen leads to increased lactate production (Murphy, 1985a). The selection of flexible poly(vinyl chloride) containers formulated with certain specific alternative plasticisers to the standard di(2-ethyl hexyl) phthalate, non vinyl polymers or redesigned thinner wall containers with an increased permeability to oxygen has made it possible to increase the period for which the platelets may be successfully stored in plastic containers. With di(2-ethyl hexyl) phthalate plasticised PVC containers, pH fell below 6.0 after 3 days in platelet concentrates containing more than 8×10^{10} platelets in 50ml, i.e. those harvested from plasma with a count exceeding 1600×10^{9}/l. In the alternative polymeric containers, there is a continuous linear production of lactate in spite of an adequate oxygen supply. The lactate is buffered by bicarbonate and there is sufficient bicarbonate in 50ml normal plasma to keep the pH above 6.0 for 7 days (Murphy 1986). A separate factor, which leads to a more rapid fall in pH and to reduction in

viability by a presently unknown mechanism, independent of pH, is failure to agitate the platelets during storage (Murphy and Gardner, 1976). In a comparison of agitation versus non-agitation during a 24 hour storage period at 22°C, recovery in vivo was 50% with agitation but only 23% without (Slichter and Harker, 1976). The method and mode of agitation have also been demonstrated as important (Murphy et al, 1982, Snyder et al, 1985). The conditions for storage of platelets in plasma at 22°C and maintaining the pH above 6.8 are best achieved by keeping the concentration of platelets below 1.5×10^9 per ml and ensuring the surface for gas exchange is greater than $400cm^2$ using normal 600 ml polymeric containers. Platelets stored at room temperature lose their ability to aggregate and therefore their haemostatic function, although these abnormalities are reversible following transfusion (Murphy and Gardner, 1976). Platelets which have been stored at room temperature probably do not produce haemostasis during the first few hours after transfusion. When platelets are stored at 4°C, their viability is poor but they are capable of producing an immediate haemostatic effect and therefore provide advantages over platelets stored at 22°C in the treatment of thrombocytopenic patients with severe bleeding. The storage of platelets in the frozen state is best achieved using dimethyl sulphoxide (DMSO) as the cryoprotective agent and employing relatively slow rates of freezing (Murphy et al, 1974). This method may be followed provided a suitable polyolefin container is employed, but it cannot be applied when using plasticised PVC platelet bags, which become brittle under liquid nitrogen storage conditions.

An important question regarding platelet storage is the extent to which in vitro measurements predict recovery in vivo, survival and haemostatic effect. Morphological changes in platelets stored at 20°C have been found to correlate well with post-transfusion viability, concentrates with the highest proportion of discoid platelets having the best survival (Kunicki et al, 1975). The disposition of the size distribution of stored platelets as determined by a Coulter counter and the extent of shape change in response to ADP, as measured with an aggregometer, were shown to correlate with survival in vivo (Murphy et al, 1984; Holme et al 1978). Measurement of morphological characteristics therefore seems to be suitable for the quality control of stored platelets. A device has been described with which the percentage of discoid platelets within a container can be determined (Fratantoni et al, 1984).

CHAPTER 3

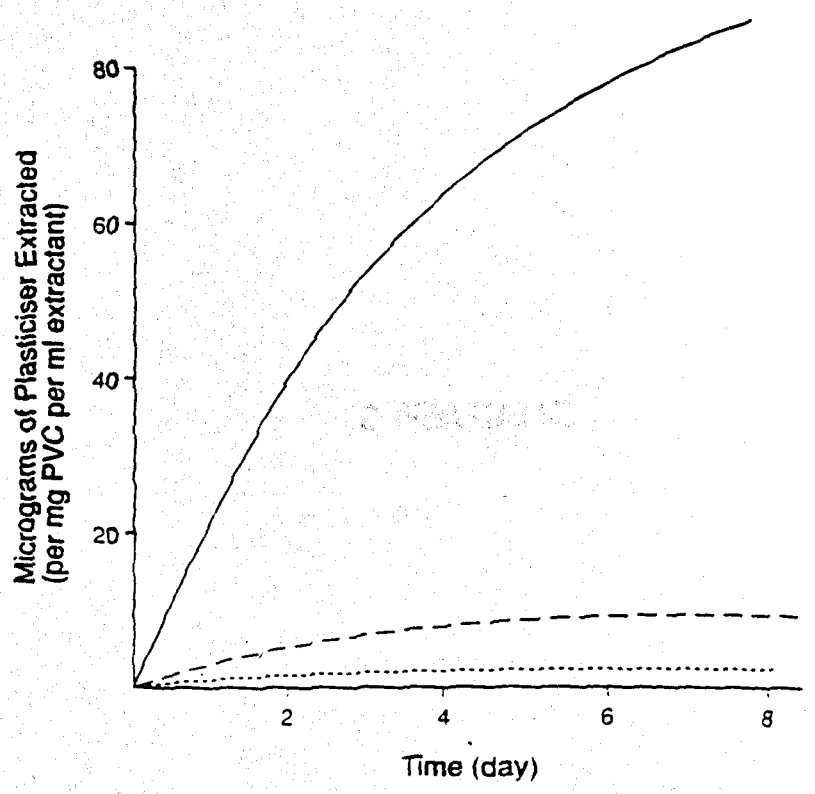


Fig. 3.1: **Micrograms of plasticisers extracted against time; immersion in human blood plasma; _____ DEHP, ——— TOTM, ... PA. (Biggs & Blass, 1987)**

CHAPTER 3.

PROPERTIES AND CHOICE OF PLASTICISED POLY(VINYL CHLORIDE)

3.1 BLOOD RESPONSE TO PLASTICISED POLY(VINYL CHLORIDE)

Flexible poly(vinyl chloride) (PVC), plasticised with di(2-ethyl hexyl) phthalate (DEHP), remains one of the most widely used polymeric biomaterials for disposable medical devices which include systems for blood collection, storage and delivery. Historically and still today the principal choice where flexible PVC is concerned, DEHP offers the benefits of overall performance, a ready availability in a suitably refined form and cost effectiveness.

Although doubts have been raised with respect to its potential toxicological properties, a CEFIC review (1985) of the available information concluded that DEHP is unlikely to pose a significant carcinogenic hazard to man. These findings were supported by BUA, an influential German advisory body on Environmentally Relevant Existing Substances which classified DEHP as non toxic in relation to acute toxicity studies. The fact remains that flexible PVC compounds plasticised with DEHP have been the subject of extensive study during the last 40 years with over 3,000 papers appearing in the literature.

While DEHP offers high resistance to extraction into aqueous systems normally encountered in general medical applications, when conditions of extended or recurring contact prevail with certain biological fluids, measurable extraction of plasticiser can result. The presence of plasticiser in a biological fluid may give rise to undesirable patient side effects. Plasticiser extraction will, in addition, decrease the flexibility of PVC components. Three examples can be quoted. Firstly cases have been reported where plasticiser extraction by stomach fluids increased the stiffness of nasogastric feeding tubes retained in position for up to 21 days to an extent whereby subsequent removal from the patient became problematic (Hayhurst & Wyman, 1975). Secondly in cases where nutritional fluids are both stored and transferred through flexible PVC, extraction of DEHP into the fatty components of such fluids was observed with subsequent transfer into the patient. The third example involves certain blood contact applications. In one instance blood may be stored for up to 21 days in flexible PVC containers and inspite of storage at low temperatures, transfer of DEHP is known to result (Jaeger & Rubin, 1972). Also, there is, in the case of haemodialysis treatment, a regular exposure of the patient's blood to PVC tubing. In one single five hour session a patient can receive up to 150 mg of DEHP (Gibson et al, 1976). Continuation of treatment at frequent intervals presents a risk of accumulation of both DEHP and its metabolites within the body. In both instances, concern is rightly expressed about the biological burden to the patient.

In recognising that DEHP is extracted into the blood stream following extended or recurring contact and could therefore give rise to undesirable side effects to the patient, alternative medically approved systems based on specially selected polymeric adipate (PA) and trioctyl trimellitate (TOTM) plasticisers have been investigated. Table 3.1 illustrates their performance in a Shore A 74 PVC formulation typically employed in the manufacture of blood tubing and fig 3.1

compares the rate of extraction by human blood plasma at ambient temperature (Biggs & Blass, 1987).

Table 3.1 : Typical physical properties of Shore A 74 PVC compounds (BS2782 Test Methods)

	Plasticiser (%)	Density (kg/m^3)	Tensile strength (MN/m^2)	Elongation (%)	Cold flex (°C)
DEHP	31.7	1.23	19.0	355	-20
PA	33.8	1.26	19.3	365	-10
TOTM	35.8	1.22	18.9	400	-20

3.1.1 Investigations of blood material interactions

The results highlighted in the preceding section lent support to the current debate regarding the suitability of plasticised PVC in blood contact applications.

However, the interactions between blood and PVC surfaces with their resulting effect upon the patient are less well documented. It may be argued that this is of equal or perhaps greater significance when contemplating the use of flexible PVC as a biomaterial.

Modern clinical treatment is highly dependent on the application of devices for the support of vital life functions. However, any surface except the endothelial lining of the vascular wall will initiate a sequence of deleterious reactions when brought into contact with blood. The most well known are associated with the activation of platelets, the plasma coagulation system, the fibrinolytic system and the complement system. In simple terms, the ability to undergo these mechanisms leading to coagulation protects us all when bleeding commences from a surface wound, but at the same time presents the surgeon with a challenge to preserve the quality and flow of blood during major surgery.

The evaluation of biomaterials attempts to correlate material characteristics with alterations in blood constituents induced by blood material contact. A basic difficulty is in the selection of parameters representative of the blood response to the artificial surface.

3.1.2 Blood compatibility evaluation procedures

A blood compatibility evaluation programme is outlined in table 3.2. The programme was based on a combination of in-vitro and ex-vivo procedures which were applied to the three blood tubing formulations previously referred to (Blass et al, 1991).

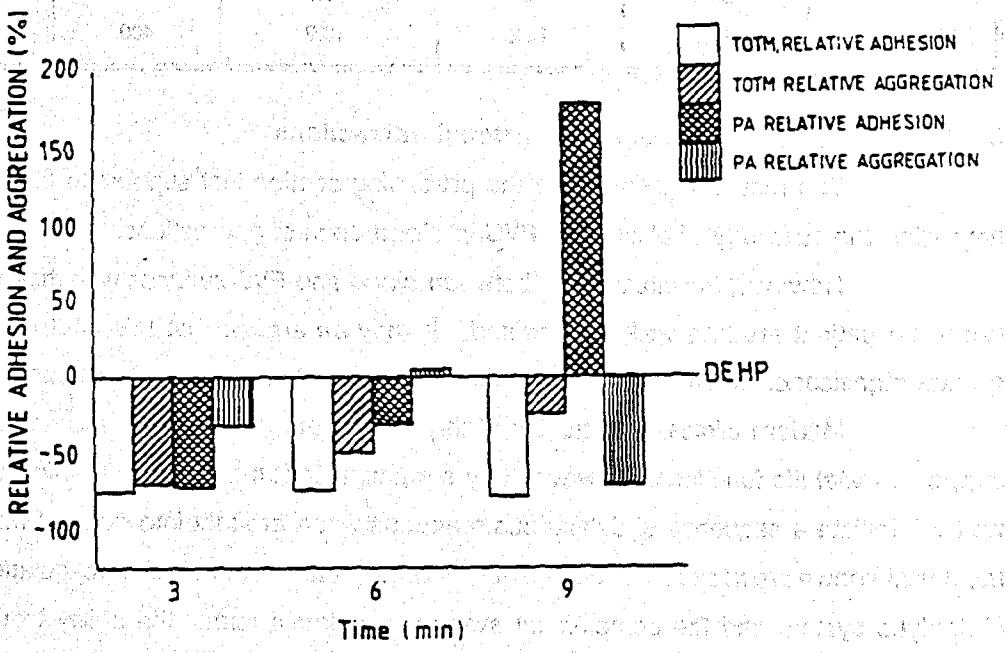


Fig. 3.2: **Platelet adhesion and aggregation results for PVC plasticised with trioctyl trimellitate (TOTM) and polymeric adipate (PA) expressed relative to the results for PVC plasticised with di(2-ethyl hexyl) phthalate (DEHP). (Courtney et al., 1989).**

Table 3.2 : Selected blood compatibility evaluation for biomaterials in artificial organs.

Feature		Parameter
In vitro	assessment in the absence or presence of antithrombotic agents	platelet adhesion platelet aggregate formation platelet release complement activation
Ex vivo	rat extracorporeal circuits	erythrocytes leucocytes platelets

3.1.2.1 *In-vitro* assessment

The *in-vitro* procedures examined platelet adhesion, platelet aggregation, platelet release and complement activation. Tests were carried out in the absence and presence of the antithrombotic agent heparin, with presterile tubings following steam, ethylene oxide and gamma irradiation treatments as appropriate.

The influence of the PVC formulations on platelet adhesion and platelet aggregation formation was determined by a modification of the Wu and Hoak method for measuring circulating platelet aggregates (Bowry et al, 1985; Courtney et al, 1987). The procedure is based on the ability of ethylene diamine tetra-acetic acid (EDTA) to disperse platelet aggregates and that of an EDTA-formalin mixture to fix any aggregates present. Platelet counts in EDTA and EDTA-formalin are made for blood before and after material contact. The procedure determines platelets lost to platelet aggregate formation and platelets lost to adhesion in the absence of platelets lost to platelet aggregate formulation.

The relationship between a biomaterial and the platelet release reaction was monitored by measuring the concentration of the platelet-specific protein, beta thromboglobulin (BTG) (Bowry et al, 1984; Travers et al, 1986). In-vitro stimulation of the release reaction occurs in response to a number of stimuli, including exposure of blood to foreign surfaces, which in this case is represented by the particular PVC formulation. A measurement of the platelet release reaction, an essential phase of platelet aggregation during coagulation, is relevant to the haemocompatibility assessment of biomaterials.

As a measure of the immune response to blood-PVC contact, complement activation was evaluated. The activation of the complement system resulting from blood-material contact is a well-established phenomenon and has been used as an index of haemocompatibility by several researchers (Chenoweth 1984; Forbes & Courtney 1987). Complement activation was determined by the measurement of the major component of the complement cascade. C3a.

The tubing under test was formed into closed loops containing a known blood volume and oscillated on a test rig for time intervals of 3, 6 and 9 minutes with the results illustrated in fig 3.2.

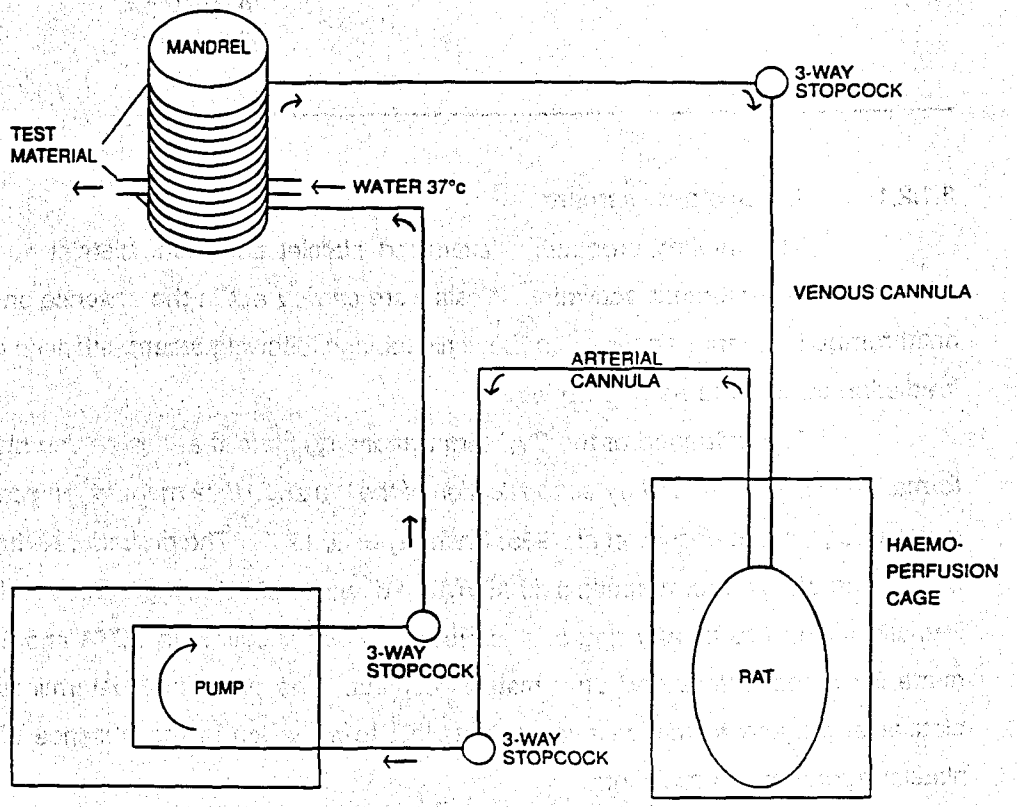


Fig. 3.3: Schematic representation of the extracorporeal circuit.

3.1.2.2 Ex-vivo assessment

The ex-vivo procedures measured levels of erythrocytes (red blood cells), leucocytes (white blood cells) and platelets in a rat extracorporeal circuit derived from a haemoperfusion circuit. (Ryan et al., 1979). Fig 3.3 illustrates a schematic representation of the extracorporeal circuit in which male Sprague-Dawley rats of mass approximately 350g were used.

Relevant features are the selection of a mass of material appropriate to the blood volume of the animal, a priming volume sufficiently small to render priming with donor blood unnecessary, elimination of the need for anaesthesia during treatment and institution of a minimum 21-h. period between cannulation and extracorporeal circulation. Small fine bore tubings (0.9mm o.d. x 0.4mm i.d.) extruded from the three compounds were used in this evaluation.

3.1.2.3 Results

Figure 3.2 compares the results obtained for TOTM and PA relative to DEHP plasticised PVC. The results determined in the absence of an anticoagulant indicate that platelet adhesion is clearly influenced by the plasticiser, with TOTM causing an improvement and PA inducing a sharp rise between 6 and 9 mins. The results for platelet aggregate formation also indicate an improvement for TOTM, with the decline for PA corresponding to the sharp rise in platelets lost to adhesion.

In the ex-vivo study, there was a gradual fall in platelet count throughout a 240 minute period for DEHP and PA as illustrated in fig 3.4. TOTM and a silicone rubber (SR) control showed a more rapid decrease between 60 and 120 minutes, with SR again showing a rapid decrease between 120 and 240 minutes. After 240 minutes, DEHP caused the least drop, followed by PA, TOTM and SR.

The in-vitro and ex-vivo blood compatibility assessment has demonstrated that plasticiser selection influences the blood response. Although interpretation of the response is complex, the data support the view that the continued use of DEHP is acceptable, but that alternative plasticisers are available (Blass et al, 1991).

3.2 COMPOSITION

The composition of the plasticised PVC formulation used in devices for blood collection, storage and delivery is determined by a combination of physical attributes and low toxicity considerations.

Flexibility, clarity and mechanical strength over the wide temperature range encountered in blood therapy are key parameters. The ease with which flexible PVC layflat extrusions or calendered films are radio frequency welded and adhesive bonded into the complex array of bags, tubings and parts, which combine to provide the typical blood collection and delivery device, has confirmed its position as the material of choice. A further important consideration is the ability of the PVC formulations to undergo successfully the various sterilisation treatments which are required, i.e. steam, ethylene oxide or irradiation by gamma rays or electron beam prior to use.

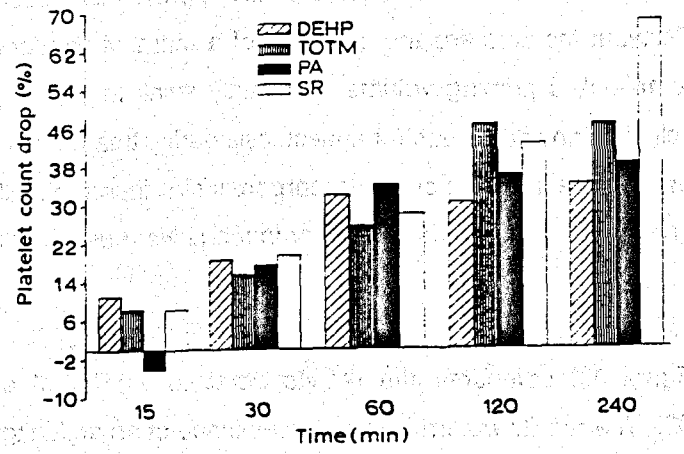


Fig. 3.4 Mean drop in platelet count from zero time. (Jones 1989)

VI.1.2.1.1 Materials based on plasticised PVC for containers for human blood, blood components, and aqueous solutions for intravenous perfusion.

.> = 55% PVC polymer
.< = 40% DEHP plasticiser
.< = 1% Zinc octanoate
.< = 1% Calcium or zinc stearate of mixture
.< = 1% NN' Di-acylethylene diamines
.< = 10% Epoxy soya or linseed oil
. No Coloring matter

Fig 3.5 European Pharmacopoeia VI. 1.2 Plastic Materials

The optimum performance is achieved by a selection of suspension PVC homopolymers exhibiting a porous particle morphology to provide a ready uptake of plasticiser during the initial dryblending sequence. The choice of additional additives used in medical grade PVC compounds is limited when compared with what is available for general purpose applications, such as building products, wire and cable sheathing. Unfortunately, there are no special listings of raw materials specifically recommended for low toxicity medical compounds, although in some cases, finished product standards define a list of permitted ingredients of the polymeric material. The choice of which raw material to use has to be based on documented lists of additives that are deemed suitable for use with polymers for food-contact applications; confirmation of the low-toxicity of an additive is necessary before it is introduced into such lists. Currently, the three major sources of this information are:

(i) British Plastics Federation (BPF)/British Industrial Biological Research Association (BIBRA) "Plastics for Food Contact Applications - a Code of Practice for Safety in Use".

(ii) US Food and Drug Administration (FDA) Code of Federal Regulations, Title 21, Chapter 1 and

(iii) West German Bundesgesundheitsamt (BGA), recommendations.

3.3 REGULATORY REQUIREMENTS AND APPROVALS

In the previous section, it was noted that while listings of additives specifically recommended for low toxicity medical compounds do not exist, examples of finished product standards relating to particular device applications are documented. Such an example is provided in fig 3.5, which illustrates the European Pharmacopoeia monograph V1.1.2.1.1 on plasticised PVC materials for containers of human blood and blood components and aqueous solutions for intravenous perfusion.

The monograph V1.1.2.1.1, like all those appearing in the European Pharmacopoeia, has to be incorporated into the individual national Pharmacopoeia of all EC Member States. However, the formulation stipulated is not mandatory but for guidance only and provision is made for the use of alternative formulations provided they are acceptable to the national regulatory authority, which in the case of the United Kingdom is the Department of Health (DOH). Furthermore, it is normal practice for a polymer raw-material supplier to divulge precise formulations to national regulatory authorities, although there is no formal approval system. A typical procedure is to file our medical compound formulations with both the DOH and the USFDA Drug Master File system in order to permit easy reference by these authorities.

- **British standards**
  - BS2464
    - Transfusion equipment for medical use
  - BS5081
    - Sterile hypodermic needles an syringes for single use
- **00H specifications**
  - TSS/B/320/-series
    - 005 PVC tubing for blood transfusion purposes
    - 008 plastics collapsible containers for blood and blood components
    - 020 plastics collapsible containers for intravenous fluids

Fig 3.6 Typical Finished Product Specifications

- **On plastic**
  - Ignition residue
  - Heavy metals
- **On aqueous extract**
  - Appearance, colour, clarity
  - Taste and smell
  - Evaporation residue
  - pH (acidity, alkalinity)
  - Oxidizable matter (reducing substances)
  - Heavy metals including lead, cadmium, tin

Fig 3.7 Typical Physico - chemical tests in UK Standards/Specifications

- **Part 1** - Guide for selection of biological methods of test
- **Test methods**
  - **Part 2** - Tissue implantation
  - **Part 3** - Systemic Toxicity/acute toxicity of extracts
  - **Part 4** - Intracutaneous reactivity of extracts
  - **Part 5** - Systemic Toxicity; Pyrogenicity of extracts
  - **Part 6** - Sensitization; delayed contact dermatitis
  - **Part 7** - Skin irritation of extracts
  - **Part 8** - Skin irritaiton of solid devices
  - **Part 9** - Eye iritation
  - **Part 10** - Cytotoxicity of extracts
  - **Part 11** - Haemolysis

Fig 3.8 British Standard 5736 - Evaluation of medical devices for biological hazards.

Other requirements for medical polymers are imposed chiefly by Pharmacopoeia, international and National standards, or for example DOH specifications relating to finished products (see fig 3.6). Although these documents are chiefly concerned with physical design and performance specifications, they also specify physico-chemical tests and, increasingly, biological test data. These requirements may even further limit the choice of ingredients used by polymer manufacturers. The typical physico-chemical tests found in British Standards (BS) or DOH specifications for medical devices are listed in fig 3.7. Some of these tests are carried out on the plastic material itself while others relate to an aqueous extract from the polymer. The extraction procedure normally simulates a steam-sterilisation process and test limits are set to permit minimum extraction of constituents from the polymer composition.

A major problem for the polymer industry is that there is as yet, no real harmonisation of test requirements between different countries. Similar tests may be required, but the precise extraction conditions may differ with respect to temperature, time and ratio of extractant to plastic. Even when the test methods are the same, different limits often exist. This means that manufacturers have to carry out different series of tests according to the ultimate destination or use of their products.

A similar problem exists with the biological test requirements and different sets of tests are required by different countries or regulatory authorities. Some of the documents concerned with these tests include :

(i) US Pharmacopoeia (National Formulary) Containers - Biological Tests (Plastics)

(ii) British Standard 5736 - Evaluation of Medical Devices for Biological Hazards (fig 3.8)

(iii) Tripartite Biocompatibility Guidance for Medical Devices (US, UK, Canada.

The US Pharmacopoeia has been used worldwide for many years because it was probably the first official document to incorporate details of biological tests for polymers. Subsequently, other countries have formulated biological test requirements, for example BS5736 (part 1) was first published in 1979 and a revised document was issued in 1989. In 1988, a route towards harmonisation was adopted by the Tripartite Guidance Document involving the DOH, Canada and the USA. The advent of the Single European Market on 1st January, 1993 has spurred on efforts in the EC, with the result that the Commission is now moving towards issuing the European Medical Device Directive, which covers the many facets of the subject under discussion for all materials used in the manufacture of health care products.

Table 3.3 PVC plasticisers used in food packaging and medical applications

NOMENCLATURE	ABBREVIATION	APPLICATION
Butyl benzyl phthalate	BBP	FC
Dibutyl phthalate	DBP	FC
Dicyclohexyl phthalate	DCHP	FC
Diethyl phthalate	DEP	FC
Di (2-ethylhexyl) phthalate	DEHP/DOP	FC
Di-isobutyl phthalate	DIBP	M
Di-isodecyl phthalate	DIDP	FC
Di-isononyl phthalate	DINP	FC
Di-isoctyl phthalate	DIOP	FC
Di-(2-ethylhexyl) adipate	DEHA/DOA	FC
Di-isodecyl adipate	DIDA	FC
Di-isononyl adipate	DINA	FC
Di-isooctyl adipate	DIOA	FC
Polymeric adipate	PA	FC/M
Diphenyl (2-ethylhexyl) phosphate	DPEHP/DPOP	FC
Di (2-ethylhexyl) azelate	DEHZ/DOZ	FC
Dibutyl sebacate	DBS	FC
Di (2-ethylhexyl) sebacate	DEHS/DOS	FC
Acetyl tributyl citrate	ATBC	FC/M
Tri (2-ethylhexyl) trimellitate	TEHP/TOTM	M
Epoxidised soya bean oil	ESBO	FC/M
Epoxidised linseed oil	ELO	FC/M

FC = food contact M = medical application

3.4 PLASTICISERS

Plasticisers are mainly organic esters with high boiling points. They are used to assist processing and impart flexibility to polymers such as PVC and vinylidene chloride copolymers (PVDC copolymers). They are in general colourless, odourless liquids, which are relatively non volatile and have a very low solubility in water. The vast majority of plasticisers are esters of phthalic acid (phthalates), with a wide variety of long chain alcohols containing up to 13 carbon atoms. The remainder are also ester or polyesters and include sebacic, or azelaic acids.

In Western Europe, about 90% of all plasticisers are used to convert rigid PVC polymers into soft, flexible and elastic materials. The remainder is used in other polymers, dispersions, coating systems and lubricants (Cadogan 1991).

The major plasticisers currently used to provide flexibility to PVC in food contact packaging and medical applications are listed in table 3.3.

It becomes immediately apparent that the number of plasticisers selected from this list and applied to medical applications is limited. A major reason is based on the costs associated with confirming their toxicological and biocompatibility characteristics combined with the limitations already referred to in connection with National Pharmacopoeia associated and regulatory bodies.

While it is not possible to be precise with respect to the demands upon those plasticisers normally employed in connection with medical device applications, the use of DEHP has and still dominates this sector after 40 years. According to the world's leading manufacture of PVC blood collection and delivery systems, less than 5% of their production involves the use of non DEHP plasticiser. Even in the case where TOTM plasticised platelet bags have shown to extend storage times from 3-5 days, sales of 3 day DEHP plasticised platelet bags still dominate the market on cost grounds.

The successful introduction of a new plasticiser will depend on its ability to demonstrate all the benefits associated with DEHP, while removing its perceived drawbacks. While a limited number of candidates have been suggested (Koerner. 1984), a new citrate based plasticiser (Labow et al, 1992) is at present receiving considerable attention. This is a subject for review in Chapter 8.

3.4.1. Plasticiser selection for medical applications

A comparison of some important plasticiser properties is shown in table 3.4.

Table 3.4 Data on Plasticisers

	DEHP	TOTM	PA
Molecular Weight	390	547	2000 approx
Viscosity (cp at 20°C)	80	300	5000 approx
Density (kgm^{-3} at 20°C)	0.983	0.986	1.075
Refractive Index	1.487	1.485	1.467
Liquid Appearance	(a)	(b)	(b)
Cost relative to DEHP	1	3.2	3.5
(a) Colourless	(b) Colourless to very pale yellow		

The influence of plasticiser selection upon the physical properties of 74° shore A hardness compounds applied in blood tubing application is given in table 3.1.

The stabilisation of these formulations was effected by a calcium-zinc system co-stabilised with epoxidised soyabean oil following the recommendations listed in the European Pharmacopoeia Monograph IV.1.2.1.1. described in fig 3.5. The choice of PVC polymer was dictated by the demands of these higher viscosity plasticisers and a suspension homopolymer specially tailored to possess the most appropriate morphological characteristics was therefore chosen.

Reduction in plasticising efficiencies by TOTM and PA in comparison with DEHP necessitated the incorporation of higher levels of these alternative plasticisers in order to achieve comparable hardness and flexibility characteristics (Blass 1990)

An important aspect of extraction resistance of these PVC compounds has been evaluated in extractants such as distilled water, vegetable oil and biological fluids (nutritional feeding solution and human blood plasma). Aqueous extraction and testing were carried out in accordance with BS 2463 methods (Transfusion Equipment for medical use), which are typical for such requirements in many national pharmacopoeia. The results, summarised in table 3.5, show that the performance of DEHP can only be matched by the selection of a specially selected TOTM plasticiser. Very few PA plasticisers, if any, are able to provide the same level of performance, although the specially selected PA complies with typical test limits.

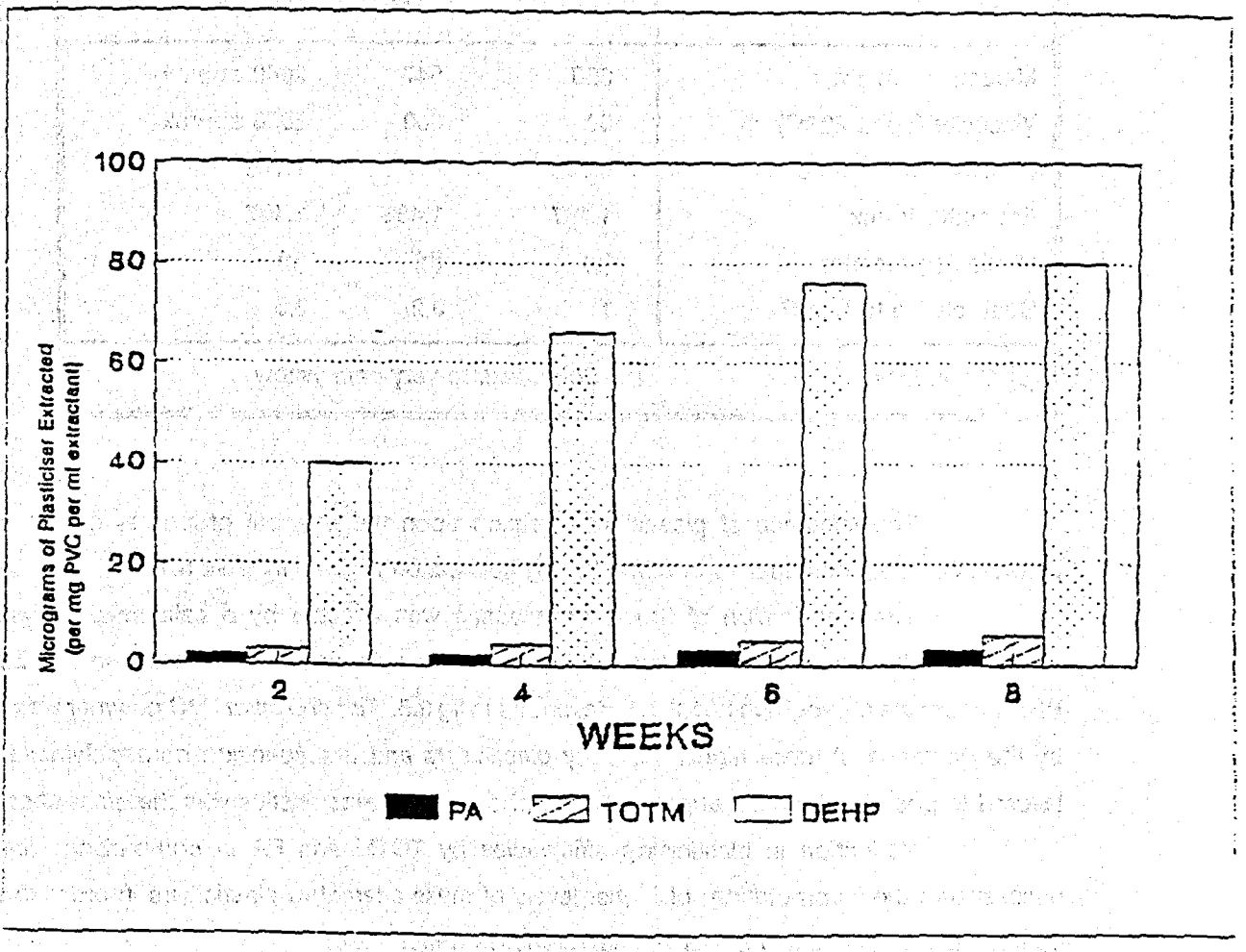


Fig 3.9 ***Radiolabelled plasticiser extraction*** **from Shore A 74 PVC compound at ambient temperature. Immersion in 10% Intralipid.**

Table 3.5 Aqueous extract tests on shore 'A 74 PVC compounds

		Plasticiser Type			
Test	Limit	DEHP	TOTM 1	TOTM 2	PA
pH	4.5 - 7.0	5.1	5.4	5.3	5.1
Evaporation residue (a)	3.00	0.45	0.30	1.20	1.70
Oxidizable matter (b)	2.00	0.30	0.30	0.90	1.50
UV absorption	0.25	0.10	0.08	0.10	0.16
(a) in mg/100 mL		(b) mL 0.01N $Na_2S_2O_3$			

Data (Biggs & Baldwin 1980) show that on immersion in oil this PA had markedly superior resistance to extraction than DEHP and TOTM, which behaved in a similar fashion to each other. After 14 days at 37°C, PVC compounds containing the last named showed up a 10% weight loss compared to no more than 1% for the PA system. In order to assess comparative performance on immersion in biological fluids, studies have been made with PVC compounds incorporating radio-labeled plasticisers (Blass, 1990). When immersed in Intralipid nutritional fluid (Kabi Pharmacia Parenterals AB) significantly less extraction occurred with TOTM and PA plasticisers, as shown in fig 3.9.

The PA was itself 4 times more resistant than the TOTM. While in practice contact time between nutritional fluid and PVC is normally of only short duration, this study was deliberately extended over several weeks in order to demonstrate the long term effects when flexible PVC components are in contact with this type of fluid, which is an example of a mixed oil in water system.

On the other hand, whole human blood and its derivative products are commonly stored in contact with PVC for extended periods and fig 3.1 illustrates a similar pattern of behaviour for the 3 plasticisers in question. The extent and nature of blood interactions with PVC are considered in subsequent chapters.

CHAPTER 4

CHAPTER 4.

POLY(VINYL CHLORIDE), PLASTICISER AND STABILISER TOXICITY

4.1 INTRODUCTION

PVC materials have been progressively applied to patient health care for almost fifty years and have therefore been the subject of a vast amount of research. In that time, considerable progress has been made in raw material selection and purification, processing and fabrication, and sterilisation to minimise any risk to patient safety. In the case of DEHP plasticiser, still predominant in flexible PVC compounds for blood contact applications, there are 3,000 published papers relating to its potential toxicological hazards.

Before considering plasticisers and the subject of blood contact, it is important to mention the concern expressed arising from researches conducted in the early 1970's by Maltoni and co-workers in Italy. They reported that the presence of high concentrations of vinyl chloride monomer led to carcinogenicity in selected animals. They linked this to a rare but lethal form of liver cancer called liver angiosarcoma found in a limited number of operators exposed to high levels of vinyl chloride monomer in the air at PVC polymerisation plants (Maltoni et al, 1984). In humans, vinyl chloride monomer (VCM) is metabolized into chloro-ethylene oxide which is believed to be the most potent mutagenic metabolite. Since 1974, the PVC industry has taken measures to reduce the risk of VCM intake by its operators and plant neighbours by means of automated production methods, closed reaction vessels and improved cleaning procedures. An average exposure to VCM not exceeding 5 ppm is now judged to give no increased incidence of cancer.

The European Pharmacopoeia requires a maximum of 1 ppm VCM in virgin PVC polymer. Modern medical grade PVC compounds are thought to contain less than 10 ppb, which is the minimum level of detection described in the EC document "VCM Analysis in Materials and Articles", 80/706/EEC.

Although the current VCM levels are not believed to pose a toxicological problem, manufacturers continue to strive for further reductions through plant modernisation and new plant installation programmes.

The use of flexible PVC compounds in blood collection, storage and delivery has provided enormous benefits to transfusion services. Without these materials present techniques of separation into blood components would be more difficult and costly to perform. However, the first reports some 25 years ago of plasticiser migration into blood products led to concern about the potential toxicological hazard associated with this occurence (Jaeger & Rubin, 1972).

Although DEHP is essentially water insoluble and only measurable in trace amounts in anticoagulant solutions or intravenous solutions stored in flexible PVC containers, levels of 50 - 70 mg/l were recorded in stored whole human blood (Jaeger & Rubin, 1970). The degree of plasticiser extracted can depend on several factors. The dominant point is the

content of lipoproteins and albumin. Whole blood constitutes a more favourable medium than washed erythrocytes, which generally have only a small content, but an affinity for plasma protein, 75% of the DEHP being associated with the lipoproteins. The temperature also plays a part in favouring migration because this is greater in blood kept at room temperature, than in blood kept at 4°C. The contact time is a favourable factor; however in practice it is less than twenty-one days, after which time the blood is rarely used. In the USA storage times are extended to forty-nine days at 4 - 6°C.

4.2 ACUTE TOXICITY

There is general agreement that DEHP is of low acute toxicity. The dose required to kill 50% of a test population (LD 50 value) of standard laboratory animals administered the chemical by various routes (oral, topical or intraperitoneal) ranges from 14,000 - 50,000 mg/kg. In fact it is lower than that of many other substances, including common salt.

However, the toxicological hazard of the direct intravenous (IV) administration of DEHP should not be ignored. This may occur as a result of, for instance, patients on haemodialysis treatment. Because DEHP is rapidly mono-de-esterified in the gut to its metabolite, mono(2-ethyl hexyl) phthalate (MEHP), it is taken up into the body primarily as the latter derivative following oral administration. Only by direct IV administration, will DEHP itself appear in the circulation, although even there the de-esterification is extensive.

When administered intravenously to rats in a form solubilized with an appropriate surfactant, DEHP has an acute LD, as low as 200 mg/kg. Death was associated with respiratory distress and was characterised by pulmonary oedema, haemorrhage and poly-morphonuclear infiltration, systems characteristic of a syndrome called shock lung. The importance of the physical form of DEHP is evident in the fact that intravenous doses of up to 500 mg/kg of DEHP dissolved in ethanol or emulsified in corn oil and water were readily tolerated and no pathological lung condition developed. However, when DEHP was solubilized directly in donor blood, lung abnormalities were seen and the LD 50 was approximately 200 mg/kg. Pulmonary injury has also been seen following in situ perfusion of the lungs in baboons and dogs with stored, banked blood.

These observations point to the importance of the physical form in which DEHP is administered, following migration into blood and to its possible effects on the lung. This suggestion is supported by reports of pulmonary insufficiency and oedema in humans following blood transfusions.

4.3 MEDIUM TERM TOXICITY

There are numerous reports of testicular atrophy in rats fed relatively high levels of DEHP, in the order of 1 - 2 g/kg/day for a period of 7 or more days. This effect is seen almost exclusively following oral administration. The IV administration of emulsions of DEHP in rats at doses up to 500 mg/kg (given as 3-hour infusions every other day for a total of 6

doses), did not cause significant atrophy. Rats appear to be a particularly sensitive model; mice are much more resistant. On the basis of species and route specificity and the high doses required, there would seem to be little to concern humans who receive DEHP contaminated blood. However, the sensitivity of humans to the testicular effects of DEHP remains to be established.

Whereas testicular atrophy clearly leads to a dramatic effect on male fertility in rats, only a few studies have investigated the effect of DEHP on female fertility. Although one study in rats indicated no effects on fertility or foetal development with oral doses of DEHP up to 1.7 g/kg daily for twelve weeks before mating (Nikonorov et al, 1973), another study in the same strain of rat at levels of DEHP as low as 195 mg/kg daily for twenty-one days before mating, reported significant decreases in litter size, neonatal body mass and neonatal survival (Onda et al, 1976). More recently, the effects on fertility were studied in female mice and a total loss of fertility was noted following DEHP at dietary levels of 0.3% for a period of seven days before mating (Reel et al, 1984). Relevant studies in humans have not been reported.

A number of studies in rats and mice have dealt with embryotoxic, foetotoxic and teratogenic effects of DEHP (Wolkowski - Tyl et al, 1983). In rats, 1% dietary DEHP intake during 21 or 20 days of gestation respectively, resulted in a reduction of foetal weight. No excess foetal death was seen. A 2% dietary level was required for a significant increase in the number of resorptions and a concomitant decrease in the number of live foetuses. However, even at the 2% DEHP level, there was no increase in the number of malformations. Mice, on the other hand, appeared more sensitive than rats. Dietary levels of 0.05% throughout pregnancy produced maternal toxicity, embryotoxicity, foetotoxicity and teratogenicity. Of particular interest in this connection, was a study in which no reproductive effects were observed in rats given IV injections of donor rat blood that had been stored in DEHP plasticised blood bags (Lewandowski and Fernandes, 1980). It was estimated that the level of DEHP ranged from 1.3 to 5.3 mg/kg per day over day 6 to day 15 of gestation.

4.4 HEPATOCARCINOGENICITY

The hepatocarcinogenicity of DEHP would appear to be an extension of a short term or subchronic effect on the liver that has long been recognised for DEHP, namely hepatomegaly. This hepatomegaly was shown by Lake et al (1975) and Moody (1978) to be due primarily to a marked increase or proliferation in a hepatic sub-cellular organelle referred to as a peroxisome. Peroxisomes have recently received considerable interest with regard to their involvement in the carcinogenic process. Basically, they represent an ancillary organelle involved in the β-oxidation of fatty acids, which results in the production of hydrogen peroxide. The generation of hydrogen peroxide and subsequent reactive oxygen species have been suggested as the causative agent in the carcinogenicity of DEHP. Two major observations are consistent with this view:

(i) DEHP, in a wide variety of tests has been shown to be non-genotoxic, i.e. it does not itself interact directly with DNA

(ii) A variety of other types of peroxisomal proliferators, notably hypolipodemic drugs such as clofibrate, are known to be hepatocarcinogens. The administration of DEHP and related plasticers or hypolipodemic drugs to non rodent species such as the marmoset, a primate considered to be metabolically closer to humans, does not lead to peroxisome proliferation and liver damage. Hypolipodemic drugs which cause peroxisome proliferation in rodents have been used by humans for many years with no ill effects.

4.5 GENOTOXICITY AND MUTAGENICITY

The non-genotoxicity of DEHP and its association with peroxisomal proliferation have profound implications for the assessment of the hazards or risks of this compound for humans. Genotoxic carcinogens are assessed routinely by non-threshold models. This approach in its ultimate form acknowledges that even a single molecule of a carcinogen will cause cancer in a defined, albeit infinitessimally small, fraction of an exposed population. On the other hand, non-genotoxic agents would presumably have a threshold effect as would be expected for the interaction of a toxic chemical with a non-DNA biological target, and would by definition have a level of exposure below which no response would be anticipated. This latter approach is the one adopted by regulatory bodies when making decisions about permitted human exposures to a variety of non-carcinogenic agents.

Two recent fundamental observations about DEHP have considerable impact on risk assessment for humans:

(i) The bulk of evidence indicates that DEHP is a tumour promoter and not a tumour initiator. (Ward et al 1987; Garvey et al, 1987). This of course implies the necessity for a threshold-based risk assessment.

(ii) There is considerable doubt that peroxisomal proliferation can occur in human hepatocytes and this calls into question the use of an inappropriate animal model in extrapolation to human risk.

If these important issues are set aside then it had been suggested that the acceptable daily intake for DEHP in humans is of the order of 10 $\mu g/kg$ or approximately 0.7 mg for a 70 kg person (Turnbull and Rodricks, 1985). If this value was even approximately correct, it would pose serious questions about the risk of DEHP exposure for humans, in view of the levels to which humans are continuously exposed to from the environment and potential contact with medical devices.

In considering the human DEHP associated hazards that arise from DEHP's migration from PVC blood bags and blood tubing, the impact of the chemical on the stored elements should not be overlooked.

DEHP has been implicated in causing decreased platelet function (Ishikawa et

al, 1983). This finding, along with the need for better gas exchange characteristics, has led to two recent developments in polymeric materials for platelet storage. A non-plasticised polyolefin plastic has been marketed for the production of platelet bags (Fenwal Division, Baxter Health Care, Round Lake, Illinois, USA). Of more immediate interest is the replacement of the DEHP plasticiser by TOTM plasticised PVC, which has extended the storage of platelet concentrates at 22°C from three to five days.

The effect upon platelet survival stored in DEHP plasticised PVC bags has been well studied and attributed to a variety of biochemical and physical changes (Rock et al, 1984).

During storage, the pH level decreases and it has been shown that below a pH of 6.1, there is a marked reduction in transfused platelet survival. With the decrease in pH, there is also an increase in the levels of plasma lactate and carbon dioxide that are present. Under these conditions, platelet viability is only 40% after 72 hours of storage (Slichter & Harker, 1976). There exists a considerable quantity of data which, show a significant relationship between the decrease in pH and the increase in carbon dioxide concentration of the plasma, and this has pointed to the importance of the removal of carbon dioxide in effecting changes during storage. The incorporation of TOTM plasticiser provides both a more permeable plastic film and reduces significantly the level of plasticiser extraction in relation to DEHP based systems. This topic is examined in greater detail in Chapter 7.

However, in the case of long-term storage of red blood cells, DEHP has been found to provide a positive benefit by enhancing membrane flexibility. Decreased flexibility of red blood cells has been shown to result directly from the loss of lipid from the membrane surface. Therefore, it has been suggested that the protection by DEHP may be due to the incorporation of the very hydrophobic DEHP into the area in the red blood cell membrane that has been vacated by the lipid. This factor could provide a good argument for the continued use of DEHP plasticised blood bags in spite of the known levels of plasticiser migration which occur.

4.6 METABOLISM AND PHARMACOKINETICS

The metabolism and pharmacokinetics or toxicokinetics of DEHP have been reviewed extensively. DEHP has been reported to be absorbed rapidly and extensively following oral administration (Woodward 1988). However, it is absorbed primarily as its mono-de-esterified metabolite, MEHP. The hydrolysis can be readily carried out by intestinal contents as well as by mucosal cells, blood and tissue lipases. A small quantity of DEHP is found in the body after oral exposure and it is found primarily as the water soluble metabolite MEHP, and its further oxidized, more water soluble products. DEHP is detected in the blood and tissues after oral administration only when massive doses have saturated the capacity of the body to hydrolyse it. MEHP itself undergoes oxidative metabolism to yield approximately thirteen or more metabolites that are excreted in the urine.

A major task has been to identify the proximate or ultimate toxic intermediate that

induces peroxisomal proliferation. At least in rats the putative proximate metabolites have been shown to be the 5-hydroxy and 5-keto derivatives of MEHP. Although these metabolites are readily formed in humans, they have not been shown to cause peroxisomal proliferation in cultured human hepatocytes, which raises the question of whether humans would be susceptible to the hepatocarcinogenic effects of DEHP seen in rodents. Furthermore, these oxidised metabolites, although unconjugated in rats, are excreted in humans primarily in the form of glucuronides. Even if human hepatic peroxisomes were capable of undergoing proliferation this raises the question of whether adequate concentrations of the appropriate reactive form of DEHP acutally occur in humans.

Taken in total, these data support the concept that DEHP could have potential toxicity for patients with possible effects on the lungs, liver and reproductive organs, even to the extent of having carcinogenic and teratogenic potential. The capacity for toxicity to lung and liver may be particularly troublesome for transfusion recipients, whose lungs are already at risk from pulmonary compromise in massive transfusion situations and hepatic disfunction from virus transmission.

However, these adverse effects have not prompted any change in blood collection, storage and delivery practice, perhaps because no clear cut human toxicity has yet been established. It is still the case that the undoubted practical benefits of DEHP plasticised PVC's for medical device applications involving blood contact outweigh the perceived disadvantages of current technology.

4.7 EVALUATION OF THE RISKS

PVC has become a very topical subject, particularly in connection with environmental concerns of domestic packaging waste disposal : recycling versus incineration, acid gas emissions and the arguments surrounding the formation of dioxins. This has to some extent spilled over into the medical device arena in particular when discussing incineration of post-hospital waste products.

In addressing the toxicology of poly(vinyl chloride) biomaterials, attention is focused on the plasticisers used to meet the range of flexibility requirements as they, by their ability to be extracted into biofluids, presented the greatest likely toxicological risk. In particular, the most widely used plasticiser DEHP was shown to be particularly labile in this respect. Animal experiments have revealed very precise toxic effects on certain biological organs or functions, which are very often characterised by a great dependence on the species in question.

The biokinetics of DEHP in rats show a number of differences compared with humans, who offer greater similarities to the monkey and to a lesser degree the hamster. The greatest number of animal experiments, and particularly those devoted to carcinogenesis studies, have been performed on rats. Irrespective of the chosen animal, an absence of an accumulation of DEHP is observed in the organism.

The prime target appears to be the liver. DEHP presents the same hypolipidemic properties as certain medical substances and like them it produces peroxisome proliferation. This effect is detectable from doses of 1000 mg/kg/day, but associated enzyme induction phenomena are seen from 100 mg/kg/day. Most researchers consider that this proliferation is only seen in rodents.

The hepatocarcinogenesis observed in rodents appears from minimum oral doses of the order of 350 mg/kg/day in rats and 750 mg/kg/day in mice. The absence of covalent bonds in the macromolecules, and the low probability of lipoperoxidation mechanisms, have directed the researches on the origin of the carcinogenesis towards the production of hydrogen peroxide and hydroxyl radicals which are responsible for substantial adverse effects on cells and capable of playing a decisive part in the development of tumors. Only rodents have been used for this type of determination. In view of the differences in sensitivity between animal species, it would seem inappropriate to extrapolate these results to humans.

Testicular atrophy and degeneration have been seen in all animal species studied. Effects are detectable from doses of 100 mg/kg/day in rats. The exact mechanisms of this action remain unclear.

Compare this analysis with observations made with hypolipidemic medicinal agents in animal models. In rodents, hypolipidemic molecules present the same toxic effects in the liver, such as proliferation of peroxisomes and hepatocellular carcinoma and atrophy in testicles. The hepatic disorders seen in animal models have not been reported in clinical trials with patients receiving 2 to 5 mg/kg/day. This species difference, seen between rodents on the one hand and primates and humans on the other cannot be attributed to different doses since these have been comparable in all species studied. The teratogenic action seems to appear only after oral administration of high doses, even the ingestion of 100 mg/kg/day as having no effect.

We may all be exposed to additives extracted from PVC food packaging films, thermoformed trays and containers on the one hand or secondly when undergoing specific health care treatment involving direct contact with PVC biomaterials. In the first case the level of extracts found in foodstuff has been shown to be extremely low. One widely held misconception concerns the migration of plasticisers from PVC clingfilms into fatty foods such as meat and cheese. The plasticiser which displays the property of clingability is not DEHP but di(2-ethylhexyl) adipate (DEHA). Experts agree that toxicity data for DEHA are far from complete. They have demonstrated however that partial substitution by polymeric adipate

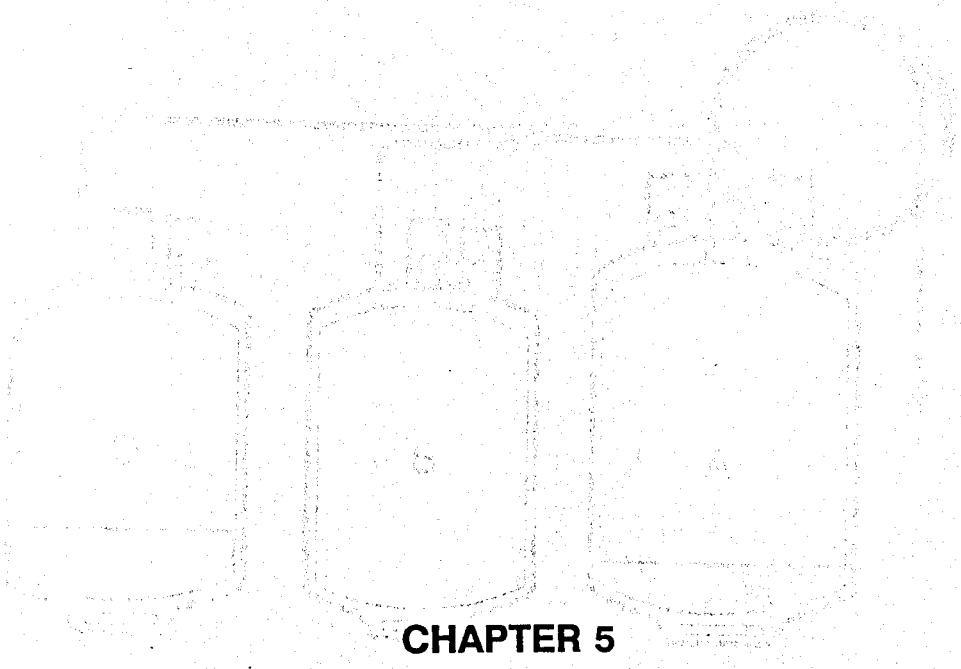
plasticisers in clingfilms has dramatically reduced the overall level of plasticiser extraction from PVC clingfilms.

In the case of exposure to PVC biomaterials as a result of health care, this occurs in specific treatments through blood transfusions or, in very restricted cases, through haemodialysis. Due to the many factors, involved it has proved difficult to specify the concentrations of DEHP in blood and blood products stored in PVC bags. As an approximation it is possible to estimate that the average content of DEHP in whole blood kept for twenty-one days at 5°C is 100 mg/l. The transfusion of one litre of blood would therefore correspond to the introduction of about 1.5 mg/kg in the case of a 70 kg adult subject. With the same reservations, a haemodialysis session is estimated to release about 200 mg of DEHP. If a frequency of three sessions per week is adopted, this represents a dose of 8.5 mg/kg/week for an adult and 400 mg/kg/year. To be able to extrapolate the results of animal experiments to man, it is customary to apply a corrective safety factor.

If the factor 100 is selected, which is an average value taking into account the accumulation of DEHP, it is seen that a subject who received one litre of blood is at the limit of the absorbed doses capable of inducing toxic effects, but the frequency of transfusion is in all cases restricted and the perfused dose or doses cannot be compared with a daily administration in any of the cases.

The subject undergoing haemodialysis on the other hand is exposed more because of the chronic nature of the treatment sessions. The excessive disparity of the values given, and the many individual factors associated in particular with a weakened renal function does not permit a precise evaluation of the risk incurred. The risk/benefit concept assumes all its importance in this case.

Finally, several aspects of PVC biomaterial toxicity merit further study. The toxicology of alternative systems such as TOTM's, polymeric adipates and citrate plasticisers which have not been referred to but have been considered in blood contact applications by analogy to well-known anticoagulants, are less well characterised than DEHP. They have shown to be less migratory in nature but this benefit could be more than outweighed by their toxicological behaviour.



CHAPTER 5

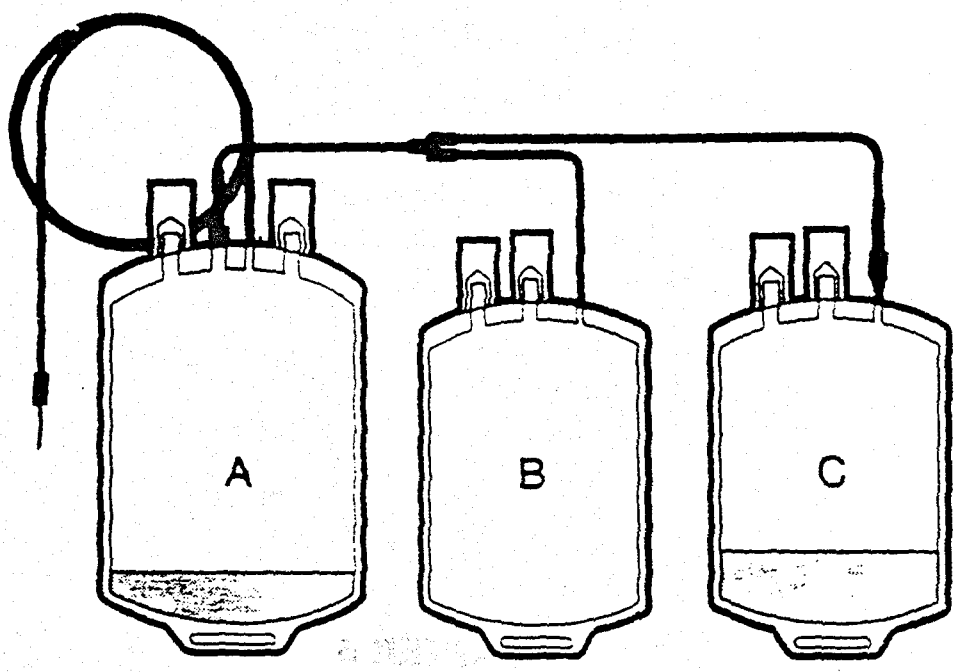


Fig. 5.1 Triple OPTIMAL ADDITIVE^{R} blood collection system as supplied. Unit A contains 63ml CPD anticoagulant. Unit C contains 100ml SAG-M solution. *(Baxter Fenwal)*

CHAPTER 5.

DISTRIBUTION OF DI (2-ETHYL HEXYL) PHTHALATE AND ITS METABOLITES IN BLOOD AND BLOOD COMPONENTS

5.1 BLOOD BAG COLLECTION SYSTEMS

Present day blood collection systems consist of multi-unit packs or containers connected by an array of flexible tubing. A wide range of designs is offered by the manufacturers to facilitate the separation of particular blood products. The basic concept can however be illustrated in the following diagram (fig 5.1), which shows a Baxter Fenwal blood collection system.

Bag A contains 63ml of citrate phosphate dextrose (CPD) preservative solution. Unit C contains 100ml SAG-M solution, which consists of saline as a diluent, adenine which extends red cell storage, glucose a sugar nutrient for red cells and mannitol who's function is to reduce the level of haemolysis. Haemolysis is the breakdown of the red cell membranes, which allows contained haemoglobin to be liberated into the surrounding plasma.

The process commences by collecting 450ml of whole blood in bag A. The next step is to centrifuge, which separates the red cells from the plasma. The supernatant plasma layer is expressed into bag B, followed by the transfer of SAG-M solution from bag C to bag A. Bag A is now sealed and disconnected and contains red cell mass suspended in SAG-M solution. The plasma in bag B can be manipulated in several ways. It can be retained in bag B, divided into 2 smaller aliquots in bags B and C, or used to prepare cryoprecipitate in bag B or cryosupernatent in bag C. Finally both bags are sealed and disconnected. Quadruple bag SAG-M systems are available and in some situations where preservation of platelets are required for extended storage at very low temperatures an alternative polyolefin bag would replace the normal PVC container.

5.2 WHOLE BLOOD AND BLOOD COMPONENTS

It has already been well documented that di (2-ethyl hexyl) phthalate plasticiser (DEHP) migrates from flexible poly (vinyl chloride) blood bags into whole blood, platelet concentrates and plasma during storage (Marcel & Noel, 1970; Jaegar & Rubin, 1972; Marcel 1973; Valeri et al, 1973; Contreras et al, 1974; Rubin & Schiffer, 1976). The level of plasticiser was shown by Peck et al (1979) to increase progressively with storage time. Results from animal studies indicate that while DEHP exhibits a low order of toxicity, its major metabolite mono (2-ethyl hexyl) phthalate (MEHP) is a more toxic compound (Villeneuve et al, 1978). Data from the United States National Cancer Institute published in 1980 suggest that DEHP induces liver tumours in rats and mice, placing emphasis on the need to monitor closely exposure to phthalic acid esters (PAE's). More recent evidence indicates that DEHP is a tumour promoter and not a tumour initiator (Ward et al, 1986, Garvey et al, 1987).

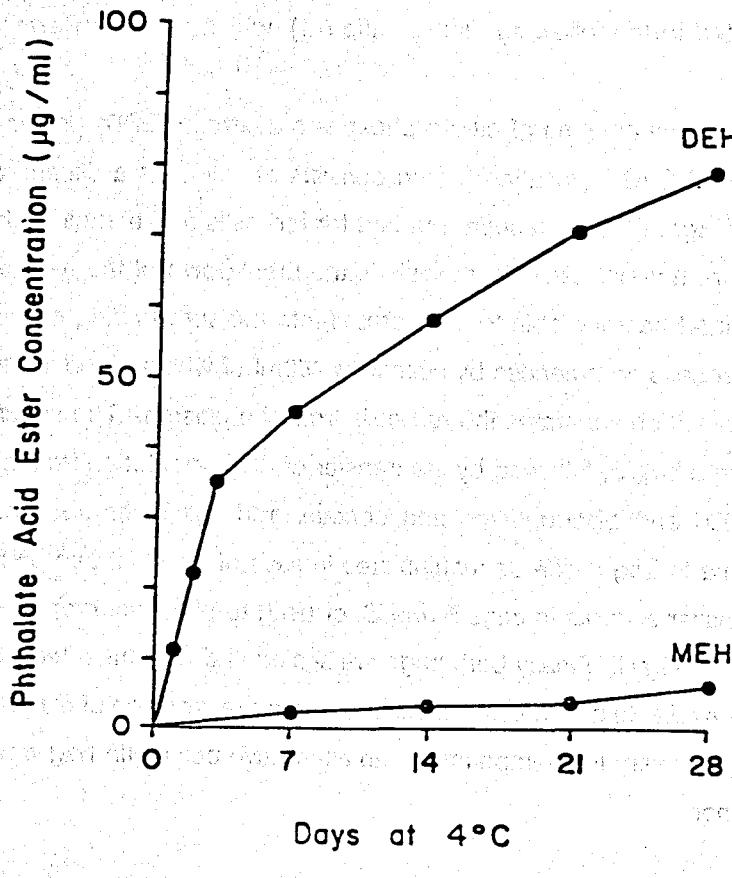


Fig 5.2 **Accumulation of DEPH and MEHP during storage of whole blood in PVC bags at 4°C.**

(Rock, et al 1986)

Studies by Rock et al (1978) and Cole et al (1979) demonstrated that during storage a plasma protein which became increasingly active at higher temperatures was responsible for the conversion of DEHP to MEHP. Therefore, depending on storage conditions, plasma used for fractionation could be contaminated by these PAEs which may precipitate with the various fractionation products. Indeed Gibbs & Coles (1975) found that four Cohn fractionations, fibrinogen, gamma-globulin, albumin, and plasma protein fraction contained low levels of DEHP. Similar results were reported by Vessman & Rietz (1974) for albumin, fibrinogen and immunoglobulin and which were prepared by a modified Cohn procedure. Low levels of MEHP were also found in fibrinogen and immunoglobulins with higher levels in albumin (Vessman & Rietz, 1978). The contamination of commercial blood products by DEHP and MEHP formed the basis of a detailed study by Cole et al (1981) and who extended by Rock et al (1986) fig 5.2. They compared the level of contamination of both DEHP and MEHP in commercially available plasma fractionation products obtained from three North American Companies and related the level of contamination to the conditions of plasma storage and transportation. Various techniques for the removal of DEHP and MEHP from albumin preparations were also investigated.

5.2.1 Materials and Methods

The blood products obtained from the three commercial companies (A, B & C) included 5% and 25% solutions of normal serum albumin (NSA), 5% plasma protein fraction, factor VIII, factor IX, immune serum globulin and Rh immune globulin. To assess the effect of storage and shipment on the presence of plasticisers, aliquots of plasma were obtained form company C after shipment from blood centres under various conditions. These samples included

(i) fresh frozen plasma

(ii) outdated, unfrozen plasma shipped at ambient temperature in the summer

(iii) outdated, unfrozen plasma shipped at ambient temperature in the winter. Shipment involved a variable period of 1-4 weeks in transit. All plasma was obtained from CPD packs

5.2.2 Results

Of the blood products tested, only factor IX from company C contained detectable levels of DEHP, as shown in table 5.1. However, MEHP was present in many of the blood products. All of the 25% NSA solutions contained detectable levels of MEHP, with the highest level recorded by company C.

Table 5.1 DEHP and MEHP levels (± SD) in blood products from three different companies

(Cole et al, 1981)

Blood product	PAE concentration, μg/ml					
	company A		company B		company C	
	MEHP	DEHP	MEHP	DEHP	MEHP	DEHP
NSA (5%)	1±0	0	trace	0	NA	NA
NSA (25%)	10±1	0	13±10	0	330±48	0
PPF (5%)	5±1	0	trace	0	NA	NA
Factor V111	1±1	0	0	0	NA	NA
Factor IX	0	0	0	0	3±2	23±16

PPF = Plasma protein fraction;
NA = not available;
trace = < μg/ml;
n = 4 for each product

To examine the effect of storage and transport of this contamination, various plasma samples were examined from company C. As shown in table 5.2 shows plasma shipped in the frozen state contained relatively low levels of DEHP and MEHP (12 and 2 μg/ml, respectively).

Table 5.2 DEHP & MEHP levels (MEHP ± SD) in plasma and NSA (25%) shipped to company C under various conditions.

(Cole, et al 1981)

Blood fraction	n	PAE concentration, μg/ml	
		MEHP	DEHP
Plasma (summer)	8	70±22	266±43
NSA (25%) (summer)	8	303±80	0
Plasma (winter)	8	44±8	219±18
NSA (25%) (winter)	8	176±63	0
Plasma (fresh frozen)	4	2±0	12±1
NSA (25%) (fresh frozen)	4	33±25	0

In contrast, outdated plasma shipped in the unfrozen state had much higher levels of DEHP. 266 μg/ml were found in the plasma shipped at ambient summer temperatures and 219 μg/ml in the plasma shipped in winter. The level of MEHP in these samples was also very high. Levels of both DEHP and MEHP in the plasma appeared to be directly related to the contamination of the 25% NSA. Table 5.2 shows the highest levels of contaminating MEHP were found in the 25% NSA made from outdated unfrozen plasma shipped at the higher summer temperatures.

5.2.3 Discussion

Contamination of plasma with DEHP and MEHP resulted in a concomitant contamination of blood products prepared by fractionation of this plasma. Products from companies A & B, which used fresh frozen plasma as starting material, did not contain DEHP, but did have low levels of MEHP in all the albumin-containing fractions, as well as in some factor VIII preparations. High levels of MEHP were found in the 25% NSA from company C which used outdated plasma as the starting material, although DEHP in this case was not detected.

These data indicated that there was a direct correlation between temperature of storage or shipment of plasma and the level of contaminating PAEs in the resultant blood products. It was apparent that MEHP had a particular affinity for albumin, since it was present in all NSA and plasma protein fraction solutions.

A plasma protein which is not associated with the platelets is responsible for the accumulation of DEHP and that for the most part the DEHP is bound to the plasma proteins (Rock et al, 1978). Where DEHP is found in any blood component, the hydrolysis product MEHP is also found to a lesser extent. The accumulation of MEHP was shown to be a direct result of the hydrolysis of DEHP by a plasma enzyme (Rock et al, 1978). This enzyme cofractionates with albumin and some commercial preparations of normal serum albumin (NSA) contain significant amounts of both DEHP and MEHP (Cole et al, 1981). Accumulation of MEHP in blood products is of concern because MEHP has been shown to be 20 times more toxic than DEHP in rats (Chu et al, 1978).

The freezing of plasma immediately after collection made it possible to prevent further extraction of DEHP or conversion to MEHP. These data differ from an earlier report by Peck et al (1979) but agree with results from red blood cell storage indicating that DEHP levels do not increase in frozen red blood cells stored for up to two years (Contreras et al, 1974), or in plasma which is frozen for 5 weeks (Vessman, 1974). These results indicate that the problem of contamination of blood fractionation products by MEHP and DEHP can best be controlled or eliminated by the use of fresh frozen plasma.

The accumulation of DEHP in stored blood is of concern for those patients receiving multiple transfusions of blood products. However, the long-term harmful side effects of this exposure has yet to be established. In animals, the acute toxicity of phthalate esters is low with oral LD_{50} values in the range 20 to 30 g/kg in mice and rats (Lawrence, 1978). In order to place this in some perspective the table 5.3 illustrates the lethal dose in mg/kg for oral application of phthalate plasticisers.

Table 5.3. Acute toxicity of various substances.

Category*	Examples of Toxic Substances	Lethal Dose in mg/kg (for oral application)
Very toxic (less than 25 mg/kg body weight)	*Botulinus Toxin* Hydrocyanic Acid Arsenic (Arsenic Oxide)	*0.00000003* 0.7 - 1.0 1.4 - 4.3
Toxic (25-200 mg/kg *body weight)*	Sodium Nitrite Barbiturates	57 - 86 47 - 143
Harmful (200 - 2000 mg/kg body weight)	Oxalic Acid Carbon Tetrachloride	375 457 - 686
Not classified as harmful (More than 2000 mg/kg body weight)	Ethanol Common Salt Plasticisers (eg DOP)	3300 7150 - 14300 More than 30000

* Based on the EEC classification levels for acute toxicity

(Plasticisers, a Consideration of their Impact on Health and the Environment. CEFIC Plasticisers Sector Group, Brussels 1989.)

Intravenous LD_{50} values in rats are in the range of 200 to 300 mg/kg. DEHP has been shown to elicit specific toxicological effects on the liver following oral administration to rats (Lawrence, 1978). A 1982 study at the National Institute of Health in the United States documented carcinogenicity in rats and mice following oral administration of large doses of DEHP (Kluwe et al, 1982). Atrophy of the testis and cessation of spermatogenesis have also been observed by Lawrence, (1978), who also detected that at the cellular level DEHP is lethal for chick embryo beating heart at a concentration of 4μg/mL. It is highly toxic to mice fibroblasts in culture and causes significant growth inhibition in human diploid fibroblasts in tissue culture (Lawrence, 1978).

These toxicity figures are however not necessarily applicable to blood and blood products; however, some typical concentrations of DEHP exposure can be calculated. Based on rates of extraction of DEHP of 0.25mg/100mL/day into whole blood stored at 4°C (Lawrence, 1978), a 70-kg man given a single a single transfusion of 3 litres of whole blood which has been kept for 3 weeks at 4°C would receive approximately 250mg/kg. Administration of various products to the 'multi transfused patient results in average exposures to DEHP in the range of 1mg per patient per day for whole blood, 0.6mg per patient per day for packed cells and 3mg per day per patient per day for platelet concentrates. (Cole et al, 1981).

Two groups of patients should be singled out for special consideration. Firstly, haemophiliacs are one of the patient populations at risk of considerable exposure, since they may receive many transfusions of blood products in a short period of time. Each cryoprecipitate

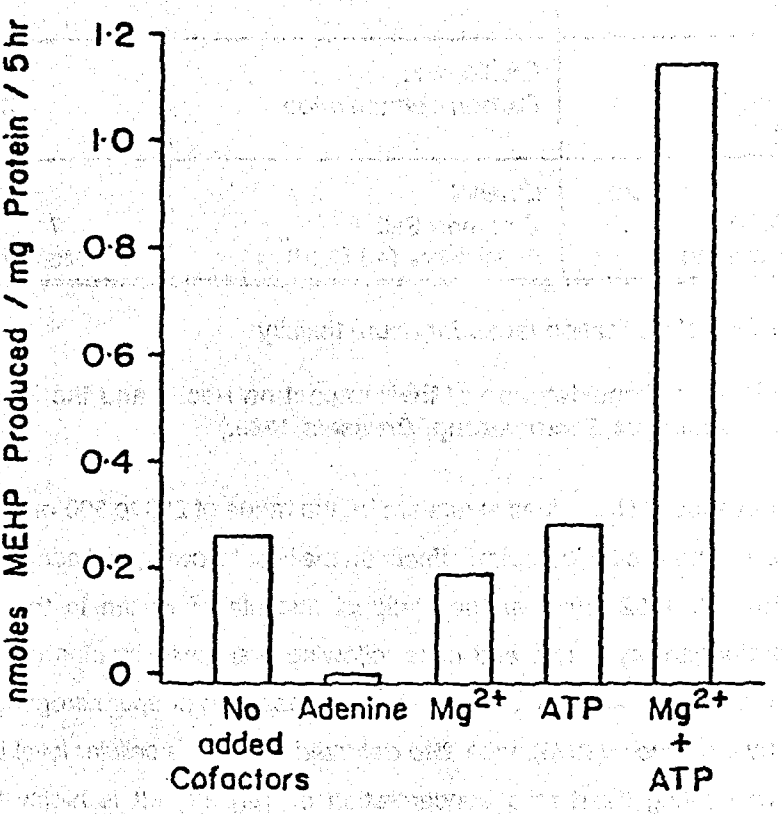


Fig 5.3 **Purified DEHP-hydrolyzing enzyme activity with added cofactors.**

(Rock et al, 1986)

contains approximately 20μg/mL of DEHP according to Rock et al (1983). The higher purity factor VIIIC concentrates contain no DEHP and less than 1μg/mL MEHP (Cole et al, 1981). Studies undertaken by Rock et al (1986) monitored DEHP blood levels before and after infusion with cryoprecipitate into haemophiliac patients, and all the DEHP was cleared from the blood 10min after infusion. Although constant exposure to DEHP can result in the induction of liver microsomal enzymes along with other structural changes Lawrence (1978), the patients in the above study showed normal liver function.

A second high risk group of patients are those undergoing renal dialysis. Several reports (Lewis 1978, Cole et al 1980) have demonstrated the extraction of 1.5mg of DEHP from haemodialysis tubing into the blood of a patient dialyzed for 5 h, with the levels peaking within the first few minutes of haemodialysis. The MEHP concentration increases gradually. Similar studies performed by Rock et al (1986) measured pre-and post - dialysis whole blood levels of DEHP in a series of 14 patients. The concentration of DEHP ranged from 1 to 14.5 μg/mL. MEHP was detected in seven of these patients before dialysis, while the post-dialysis blood samples of all 14 patients contained 0.2-4μ/mL of the compound. The high levels of DEHP in the predialysis samples in some patients were found to be due to plasticiser extraction from the Terumo AVF extension set. Their study showed that DEHP was extracted from the artificial kidney and was detectable in blood after only one passage though the dialysis machine. However DEHP was rapidly removed from the circulatory system as none was found in blood after 15 minutes. MEHP also appeared in the blood after one passage through the artificial kidney, indicating that DEHP was rapidly converted to MEHP, the level of which remained high after 15 min.

If daily plasma exchange is required for 8 days, as is often the case, the total short term exposure would be 29mg DEHP. Exposure approaching this value could occur for cytopheresis or plasmapheresis donors thereby putting the normal donor population at risk.

The presence of MEHP is detected in blood alongside DEHP following contact with DEHP plasticised PVC medical devices. The characteristics and site of this metabolic conversion were studies by Rock et al (1986). They found one site for this reaction to be plasma. A soluble enzyme which converts DEHP to MEHP was partially purified by high speed centrifugation of platelet poor plasma (PPP). The enzyme responsible for the conversion had a pH optimum of 7.2 and a molecular weight of 50,000 to 100,000; it appeared to exist in a complex with the oxidation system that further degraded MEHP. In its partially purified states, Mg^{2+} and ATP stimulated the activity, whereas adenine (0.13 mM) and detergents inhibited it as illustrated in Figure 5.3. The enzyme was freed of 95% of the plasma albumin by passage through an Affigel Blue column; however polyacrylamide gel electrophoresis of the partially purified enzyme still indicated a significant contamination by albumin.

CHAPTER 6

CHAPTER 6:

THE INFLUENCE OF PLASTICISED POLY(VINYL CHLORIDE) ON THE SURVIVAL OF STORED RED BLOOD CELLS

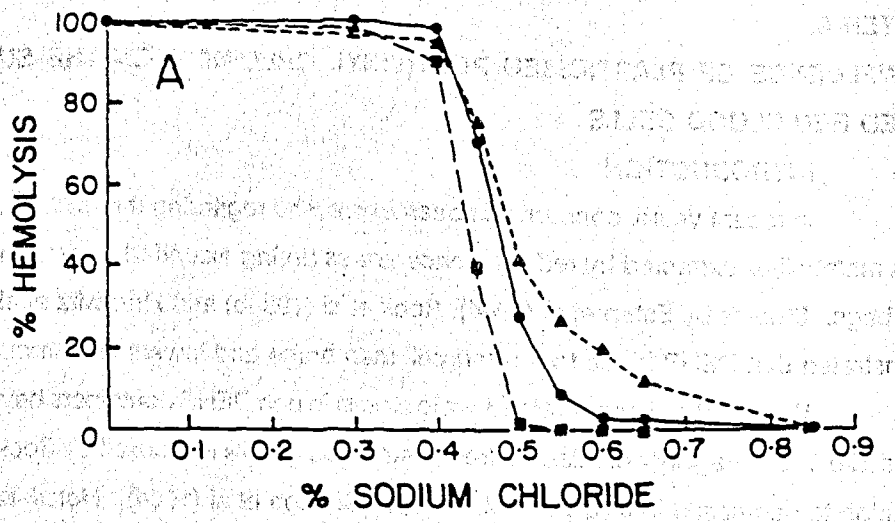
6.1 INTRODUCTION

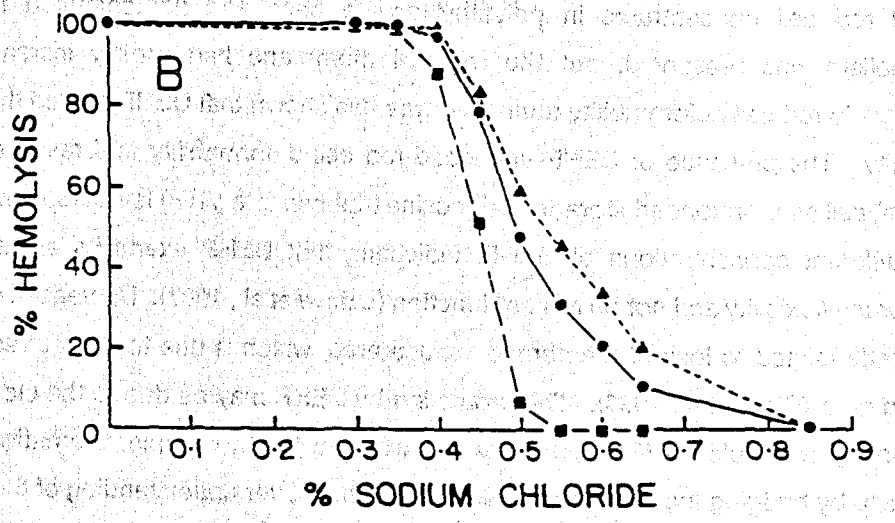
In recent years, concern has been expressed regarding the toxic effects of DEHP and its metabolites extracted by red cell concentrates during extended storage in flexible PVC blood bags. Studies by Estep et al (1984), Rock et al (1984b) and Horowitz et al (1985) have demonstrated that DEHP binds to the red cell membrane and lowers its osmotic fragility.

When red cell concentrates were stored in non DEHP containers beyond 21 days, increased osmotic fragility and plasma-free haemoglobin were measured by Rock et al (1984), in addition to decreased in-vivo survival times, AuBuchon et al (1984). Horowitz et al (1985) stored red cell concentrates in polyethylacrylate bags and demonstrated that red cell metabolism was preserved, but the red cell membrane had greatly increased fragility. Changes in red cell deformability during storage has shown that DEHP altered the membrane flexibility. The presence of DEHP enhanced red cell deformability and resulted in a "more flexible" cell after prolonged storage. Adenosine triphosphate (ATP) levels remained unaltered with different concentrations of DEHP, indicating that DEHP exerts its effect directly on membrane flexibility and not on red cell function (Labow et al , 1987). Decreased deformability is directly related to loss of membrane surface area, which is due to the altered cholesterol to lipid ratio (Clark et al, 1983). The protection by DEHP may be due to the incorporation of the very hydrophobic DEHP into the area that was vacated by the lipid, alternatively the DEHP may help by bridging the asymmetric bilayers. With a better understanding of the mechanism of this DEHP protection it might be possible to introduce an alternative stabilising lipophilic compound demonstrating a reduced degree of toxicity performance.

6.2 INCORPORATION OF DEHP PLASTICISER INTO RED CELLS DURING STORAGE

The association of DEHP and stored red cells using ^{14}C - DEHP as a marker was examined by Rock et al (1984). Their investigations showed that the DEHP was taken up by both the membrane and cytosol fractions of stored red cells (RBC'S). In addition the presence of DEHP correlated with a protective effect on red cell fragility. Their results illustrated in table 6.1 demonstrated that 28% of available ^{14}C -DEHP became bound immediately to sites in both the membrane and cytosol fractions of the red cells, and that the total amount and distribution of ^{14}C-DEHP did not change significantly over a 7 day storage period of 4°C.





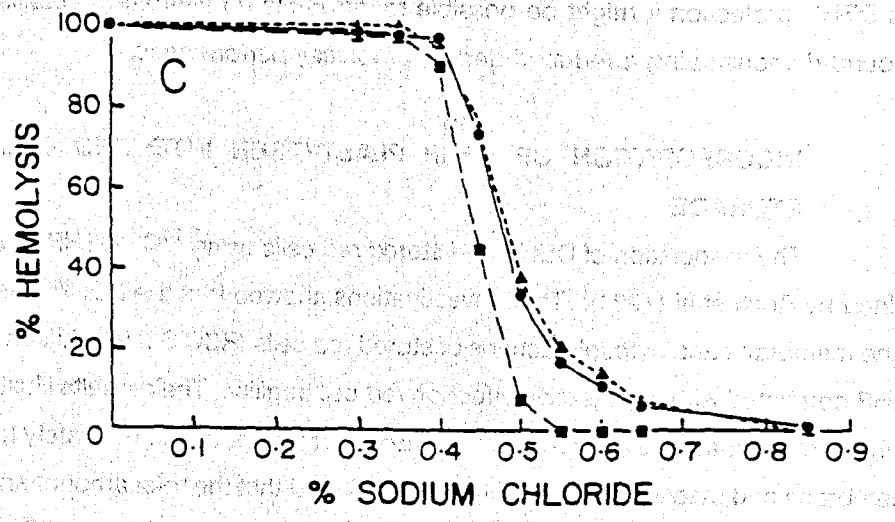


Fig 6.1 **Red cell concentrate were prepared by adding either fresh, plasma or plasma enriched with DEHP to packed RBC's. Red cell osmotic fragility was determined on days 0, 21, & 35. Red cell concentrates (A) containing DEHP enriched plasma were stored in polyolef in bags; (B) contained fresh plasma in polyolefin bags; and (C) contained fresh plasma in PVC bags, (n=3), ▪--▪, day O, ●--●, day 21, ▲--▲, day 35 of storage.**

The effect of DEHP on red cell osmotic fragility when concentrates were prepared with either fresh plasma or plasma enriched with DEHP and stored in polyolefin or PVC blood bags is shown in fig 6.1.

Table 6.1 Percent association of ^{14}C-DEHP with red cells

Samples	Days Stored at 4°C	
	0	7
Red cell concentrate before washing	100.0 ± 0*	100.0 ± 0
Supernatant wash	35.9 ± 1.6(a)[1]	47.9 ± 4.6(b)[1]
Platelet pellet from wash	6.3 ± 2.0	10.0 ± 3.4
Washed RBCs	28.0 ± 2.4(c)[2]	24.6 ± 5.5(d)[2]
RBC cytosol	14.7 ± 0.8	13.0 ± 0.8
RBC membrane	11.6 ± 1.2	11.7 ± 2.0

* Mean ± SD (n=4)

[1] a:b are significantly different at $p < 0.05$

[2] c:d are not significantly different at $p < 0.05$ (unpaired *t* test).

The red cell concentrates were diluted with fresh plasma prepared from blood drawn into CPD to a haematocrit of 40%, then incubated for 1 hour at 37°C prior to testing. RBC osmotic fragility was determined by using Becton Dickinson Unopette test kits according to manufacturers instructions. The percentage haemolysis produced by each concentration of sodium chloride was then plotted.

At 21 days, the osmotic fragility of series A (prepared with plasma enriched with DEHP and stored in polyolefin bags) and series C (prepared with fresh plasma and stored in PVC bags) were not markedly different from each other, but were considerably better than series B (prepared with fresh plasma and stored in polyolefin bags). By the 35th day, however the osmotic fragility of series A red cells had increased, whereas those in series C remained unchanged.

The cell concentrates were analysed for DEHP content. On day O, the amounts for DEHP were as follows: series A, 97.2μ per ml; series B, 30.0μg per ml; and series C, 34.2μg per ml. The low levels of DEHP in series B & C reflect the brief exposure of the packed RBC's and plasma to DEHP since they were collected in PVC bags containing DEHP. By day 35, the DEHP concentration in the PVC bags (series C) had increased to 418.3μg per ml. The pH was measured on days 0, 21 and 35 and showed no significant difference between the samples in Series A, B or C on any given day.

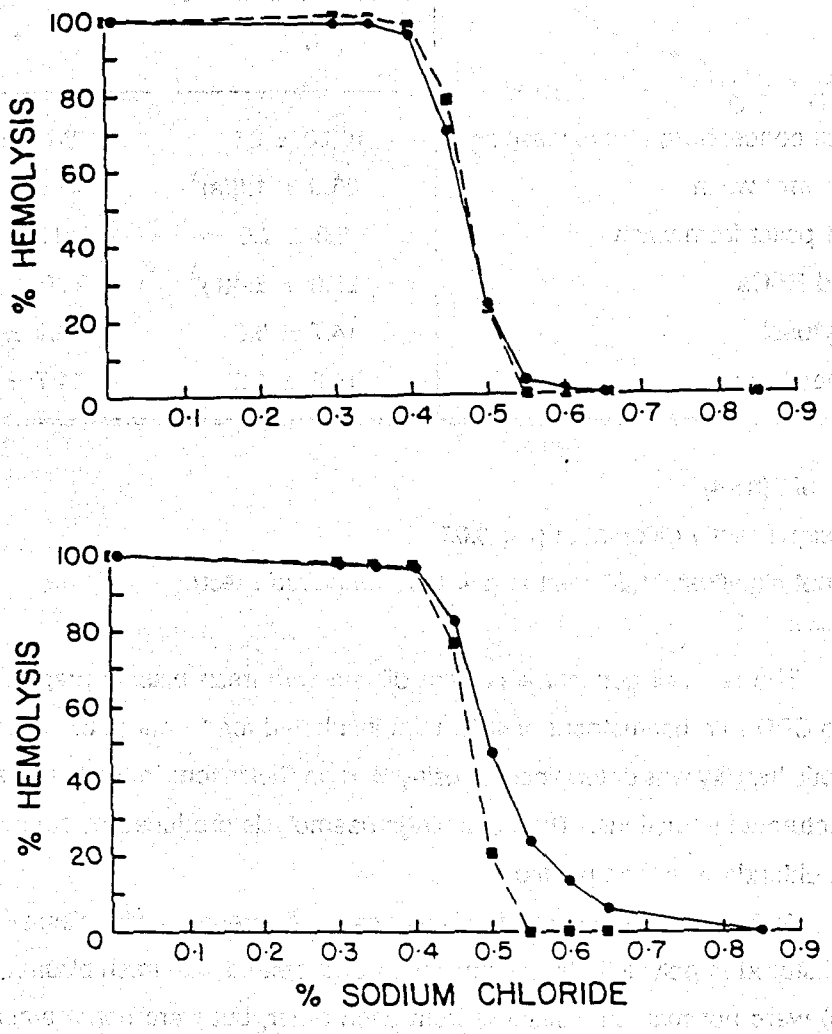


Fig. 6.2 **Osmotic fragility tests were done red cell concentrates stored in glass at 4°C for 21 days. The concentrates stored were made with freshly packed RBC's and either plasma containing DEHP (top) or not containing DEHP (bottom), (n=3). ▪—▪, day O storage, ●—●, day 21 storage.**

(Rock et al, 1984)

Table 6.2 Plasma haemoglobin values of stored red cell concentrates*

	Red Cell Concentrates	Plasma Haemoglobin, mg per dl		
		Day 0	Day 21	Day 35
Series:				
A	Prepared with DEHP plasma, stored in polyolefin packs	5.7 ± 2.0[1]	30.3 ± 3.1	118.3 ± 38.5
B	Prepared with fresh plasma, stored in polyolefin packs	26.7 ± 3.6	73.3 ± 13.4	181.7 ± 70.6
C	Prepared with fresh plasma, stored in PVC packs	21.2 ± 6.5	42.3 ± 8.4	90.3 ± 20.8

* The red cell concentrates were prepared from pooled RBCs and stored at 4°C. Immediately prior to assay, they were diluted to a hematrocrit of 40 percent with fresh plasma.

[1] Mean ± SD (n = 3).

The results in table 6.2 demonstrated that plasma haemoglobin increased within each series over 35 days, but there was less haemoglobin in Series C (stored in PVC bags) on day 35 (90.3 mg/dl) than in either of the two red cell concentrates stored in polyolefin bags (118.3 mg/dl, 181.7 mg/dl). The concentrates prepared with fresh plasma and stored in polyolefin bags (B) had the highest concentration of plasma haemoglobin both on day 21 and 35. The very low level of haemoglobin (5.7 mg/dl) on day 0 occurred in Series A samples, in which a significant amount of DEHP was already present.

Small volumes of red cell concentrates prepared with packed RBC's and either plasma enriched with DEHP, or control plasma not containing DEHP, were stored for 21 days in glass tubes. There was increased osmotic fragility of cells stored without DEHP for 21 days as shown in fig 6.2. The level of DEHP in the cell concentrates was 50.8 ± 1.4μg per ml on day 0, and 40.0 ± 0μg per ml on day 21. There was no detectable DEHP in the control samples.

The results presented by Rock et al (1984) clearly demonstrated that when red cell concentrates are stored with ^{14}C-DEHP, a considerable amount of the ^{14}C-DEHP becomes associated with the red cell membrane and cytosol fractions. In contrast earlier reports had not found significant amounts of DEHP taken up by the red cells (Jaegar & Rubin 1972, Valeri et al 1973, Contreras et al 1974). This discrepancy may be explained by the fact that earlier studies examined red cells stored as whole blood, not red cell concentrates. Since DEHP is taken up by the lipid fraction of plasma, much of the DEHP in the former experiments may preferentially have bound to the plasma lipoproteins. In the case of red cell concentrates, limited plasma is available, therefore favouring association of the DEHP with RBC's. This difference has important implications since it is red cell concentrates rather than whole blood which are stored in the majority of blood banks. Previous estimated levels of plasticisers received by patients have probably been understated since the calculations were based on primarily plasma concentrations, and ignored the uptake by RBC's.

While the improved in vitro stability of red cells in the presence of DEHP observed previously by Stern & Carman (1980) has therefore been confirmed, it is recognised that laboratory tests such as changes in osmotic fragility and the degree of spontaneous lysis do not correlate with in vivo viability of red cells (Mollison, 1983). However osmotic fragility has been shown to be a sensitive model for assessing red cell stability in vitro (Danon et al, 1964; Valeri et al, 1965; Dern et al, 1966).

The possibility was also considered that rather than DEHP displaying a beneficial influence on the red cells, unidentified factors in the polyolefin bags might affect them adversely. However, when red cell concentrates were stored in glass with and without DEHP the results confirmed the findings that DEHP stabilised the red cells. This effect was most pronounced after 35 days indicating that long-term storage in the absence of DEHP severely compromised the red cells (Rock et al, 1984).

6.3 CHARACTERISATION OF RED BLOOD CELL QUALITY DURING REFRIGERATED STORAGE OF WHOLE BLOOD CONTAINING DEHP PLASTICISER.

Estep et al (1984) set out to determine whether DEHP affects red blood cell integrity by comparing cell morphology, plasma haemoglobin accumulation, microvesicle production, and the concentration of intracellular metabolites and electrolytes of erythrocytes from blood stored at 4°C in the presence and absence of DEHP.

When sufficient emulsified DEHP was mixed with whole blood to give a final concentration of 300μg/ml, plasma haemoglobin accumulation was reduced by an average of 70%, the percentage of cells exhibiting normal morphology was enhanced 20-fold, and the volume of microvesicles released from red blood cells was reduced by 50% after 35 days of refrigerated storage compared to the values obtained from corresponding samples stored without added DEHP, as illustrated in table 6.3.

Table 6.3. Plasma Haemoglobin Concentration and Erythrocyte Morphology in Whole Blood as a Function of Storage Duration at 4°C With and Without 300 μg/mL Emulsified DEHP

Parameter	Blood Additive	Parameter Value					
		Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Plasma hemoglobin concentration (mg/dL)	Buffer solution	7±2	18±6	32±8	60±17	142±34	
	Buffer solution + 300 μg/ml emulsifier	5±1	14±5	27±8	46±12	74±28	99±37
	Buffer solution + 300μg/mL emulsifier + 300 μg/mL DEHP	8±3	9±5	11±2	17±6	22±6	34±13
Percent cells of normal morphology	Buffer solution	74±2	26±7	17±7	27±7	26±22	2±1
	Buffer solution + 300 μg/mL emulsifier	73±7	38±11	30±4	35±21	32±19	5±3
	Buffer solution 300 μg/mL emulsifier + 300 μg/mL DEHP	90±6	74±14	64±13	68±13	62±15	43±17

Results are expressed as the mean ± SD of three samples stored in polypropylene tubes.

The erythrocyte stability observed in the presence of DEHP was also not attributable to its hydrolysed metabolites namely mono (2-ethylhexyl) phthalate (MEHP) or 2-ethylhexanol (EH) as shown in table 6.4.

Table 6.4 Plasma Haemoglobin Accumulation and RBC Morphology in Whole Blood Stored for 35 Days at 4°C With Emulsified DEHP or DEHP Hydrolysis Products

Blood Additive	Plasma Haemoglobin Content (mg/dL)	Percent Cells of Normal Morphology
Buffer solution	142 ± 35	2 ± 1
Buffer solution + 300 μg/mL emulsifier	9 ± 37	4 ± 3
Buffer solution + 300 μg/mL emulsifier + 60μg/mL MEHP	82 ± 27	3 ± 1
Buffer solution + 300 μ/mL emulsifier + 30μg/mL EH	77 ± 67	4 ± 2
Buffer solution + 300 μg/mL emulsifier + 60μg/mL MEHP 30 μg/mL EH	78 ± 33	4 ± 2
Buffer solution + 300 μg/mL emulsifier + 300 μg/mL DEHP	34 ± 13	43 ± 17

Results are expressed as the mean ± SD of three samples stored in polypropylene tubes.

Table 6.6 **Effect of 300 μg/mL DEHP on Red Blood Cells Metabolites, Electrolytes, and Microvesicles Formation During the Refrigeration Storage of Whole Blood**

		Parameter Value					
Parameter	Blood Additive	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
ATP concentration (μmol/gHb)	300μg/mL emulsifier	4.4±0.6	3.7±0.6	3.5±0.6	3.0±0.7	2.7±0.8	2.1±0.7
	300μg/mL emulsifier - 300 μg/mLDEHP	4.5±0.4	3.8±0.3	3.4±0.5	3.4±0.5	3.0±0.8	2.2±0.7
2,3-DGP Concentration (μmol/gHb)	300μg/mL emulsifier	9.9±2.6	3.5±1.4	3.5±1.4	1.0±0.3	0.4±0.5	0.4±0.5
	300μg/mL emulsifier - 300 μg/mLDEHP	9.1±1.1	10.5±2.5	4.4±1.8	1.7±0.3	0.7±0.4	0.4±0.1
Na± concentration (μmol/L cells)	300μg/mL emulsifier	10.6±1.5	24.6±1.2	33.6±2.4	41.4±3.4	49.6±6.2	51.3±3.2
	300μg/mL emulsifier - 3000 μg/mLDEHP	10.2±0.8	25.1±2.3	35.1±2.0	41.8±3.4	49.1±3.1	53.4±4.5
K± concentration (μmol/L cells)	300μg/mL emulsifier	106.9±4.2	87.0±2.1	71.9±3.2	66.2±1.9	61.3±3.3	57.4±3.2
	300μg/mL emulsifier - 300 μg/mLDEHP	98.3±8.1	84.3±1.7	70.5±3.7	63.2±2.1	56.7±4.4	53.7±2.0
Plasma Hb in microvesicles (mg/d:)	300 μg/mL emulsifier						17.0±7.0
	300 μg/mL emulsifier - 300 μg/mL DEHP						7.0±4.0

Results are expressed as the mean ± Sd of four samples. Blood was stored in transferd packs from TOTM plasticized PVC

*P<.02 by paired comparison with control samples.

When sufficient MEHP and/or EH were added to blood to stimulate the degree of hydrolysis typically observed during a 35 day storage period (approximately 20% of a 300μg/ml dose of DEHP as assessed by GLC), there was a 17% to 22% reduction in plasma haemoglobin accumulation relative to the emulsifier control samples, compared to the corresponding 66% decrease observed when DEHP was added. Additionally, there was no improvement in the preservation of normal erythrocyte morphology in the presence of MEHP and/or EH in contrast to the noticeable difference observed in the DEHP containing samples.

Estep et al (1984) went on to demonstrate similar effects regardless of whether blood was stored in polypropylene containers as in the forgoing cases, or in flexible PVC bags plasticised with tri (2-ethylhexyl) trimellitate (TOTM) and with DEHP solubilized by a variety of emulsifiers (table 6.5). The use of TOTM plasticised PVC compounds has become more widespread in recent years for the short term storage of platelet preparations, and is considered in more detail chapter 7.

Table 6.5 Plasma Haemoglobin Content and Erythrocyte Morphology After the Refrigerated Storage of Whole Blood for 35 Days in TEHTM Plasticized Packs With and Without 300 μg/mL Emulsified DEHP

Blood Additive	Plasma Haemoglobin Content (mg/dL. Mean = SD)	Percent Cells of Normal Morphology (Mean ± SD)
Buffer solution	71 ± 32 (n - 3)	4 ± 1 (n - 3)
Buffer solution + 300 μg/mL emulsifier	51 ± 28 (n - 3)	12 ± 4 (n - 3)
Buffer solution + 300 μg/mL emulsifier + 300 μg/mL DEHP	32 ± 24 (n - 6)*	45 ± 10 (n - 6)*

* $p < .05$ by paired comparison with either buffer or emulsifier control samples.

When 300μg/ml DEHP were added to stored blood containing erythrocytes predominately in the echinocyte conformation many of the cells reverted to the normal discoid morphology. The addition of this quantity of DEHP to blood had no significant effect on the path of storage-induced changes in erythrocyte adenosine triphosphate (ATP), 2,3-diphosphoglycerate (2,3-DPG), sodium or potassium concentrations (table 6.6). The data presented are consistent with the hypothesis that DEHP inhibits the deterioration of the red blood cell membrane that results from the refrigerated storage of whole blood.

Further support for this view was provided in studies undertaken at the New York Blood Center where an evaluation was conducted of poly(ethylene-co-ethyl acrylate) (EEA) as an alternative blood bag material to DEHP plasticised PVC (Horowitz et al, 1985). Blood was collected initially into PVC blood bags containing CPD anticoagulant and then each unit was subdivided aseptically among the storage containers being compared. Storage containers were formed from two sheets of sterile plastic film held together by specifically designed clamping

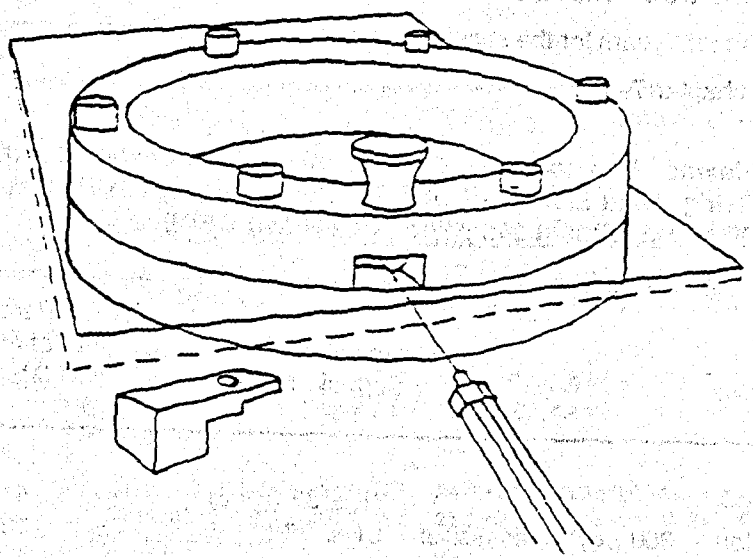


Fig. 6.3 **Schematic drawing of clamping ring. Two sheets of sterile plastic are aseptically placed between the two rings, which are then clamped together by means of 6 point bolts. The bolts lay to the outside of the raised clamping surface. Also pictured are the inlet enclosure and syringe used for the introduction and sampling of blood. The inner diameter of the ring is 3.5in.**

(Horowitz et al. 1985)

rings with an internal diameter of 8.89 cm as shown in fig 6.3. The provision of these clamping rings allowed the construction of hybrid bags with different plastics forming each of the two walls, permitted circular inserts of test films between the walls prior to clamping and ensured uniform geometry and seam construction. The introduction of 35 ml of blood was effected as shown into each container and stored at 4°C for 21 days. Plasma haemoglobin was determined following its conversion to cyanmethemoglobin, and erythrocyte osmotic fragility was determined by measuring the haemoglobin released after incubation for 30 min with hypotonic solutions of buffered sodium chloride. Erythrocytes stored as whole blood in containers made from EEA as compared with DEHP plasticised PVC (table 6.7) exhibited higher levels of plasma haemoglobin and a higher susceptibility to osmotic lysis. Introduction of a single PVC surface into the storage chamber, either as a wall or as an insert, caused a distinct incremental increase in erythrocyte stability as judged by measurements of plasma haemoglobin and erythrocyte osmotic fragility. Smaller incremental increases in erythrocyte stability were observed with additional PVC surfaces.

Table 6.7 Plastic effects on erythrocyte stability [1]

Code	Container walls	Insert	Exposure to (surfaces)		Plasma hemoglobin[2] mg/dl	Osmotic fragility$_3$ % lysis
			EEA	PVC		
a	EEA-EEA	EEA	3	0	109 ± 54	20 ± 9
b	EEA-EEA	-	2	0	104 ± 48	19 ± 9
c	EEA-EEA	PVC	2	1	55 ± 36	9 ± 4
d	EEA-PVC	EEA	2	1	56 ± 27	8 ± 5
e	EEA-PVC	-	1	1	51 ± 29	7 ± 4
f	EEA-PVC	PVC	1	2	36 ± 21	5 ± 3
g	PVC-PVC	EEA	1	2	41 ± 31	5 ± 3
h	PVC-PVC	-	0	2	35 ± 28	4 ± 2
i	PVC-PVC	PVC	0	3	31 ± 19	3 ± 2

[1] 6 units of whole blood were each subdivided among the containers described (35 ml/container) and stored for 21 days at 4°C. The area of a single outer wall was 9.6in^2. The area of the insert was also 9.6 in^2, counting both of its sides.

[2] For purposes of comparison, whole blood contains approximately 15 g/dl.

[3] Lysis on exposure to 0.55% NaCl.

To demonstrate the possible effect of DEHP being responsible for erythrocyte stabilisation during storage, containers were made with EEA which incorporated 0, 3, 6 and 9% DEHP (w/w) during the film extrusion process. Osmotic fragility of erythrocytes on exposure to 0.50% sodium chloride solution proved to be a sensitive marker of erythrocyte integrity after a 21 day storage period, as demonstrated in table 6.8. Blood stored in EEA containers without DEHP exhibited a higher erythrocyte osmotic fragility than blood stored in PVC. Incorporation of 3, 6 or 9% DEHP into EEA reduced the erythrocyte osmotic fragility as compared to the unmodified EEA containers. The higher the DEHP content, the lower the erythrocyte osmotic fragility, although the differences were too small to be statistically significant. No reasons were given as to why the EEA formulated with 9% DEHP improved the stability of erythrocytes over that observed on storage in the PVC control.

Table 6.8 Storage in containers made form EEA formulated with DEHP[1]

Container	Lysis with 0.50% NaCl, %
EEA, no DEHP	51.3 ± 14.3
EEA, 3% DEHP	33.4 ± 18.6
EEA, 6% DEHP	26.1 ± 8.9
EEA, 9% DEHP	23.6 ± 10.6
PVC control	30.1 ± 10.0

[1] Units of whole blood in CPD were each distributed among the containers listed and stored at 4°C for 21 days.

(Horowitz et al, 1985)

6.4 THE INFLUENCE OF DEHP ON RED CELL DEFORMABILITY

It is widely accepted that impaired red cell deformability can cause a reduction in cell survival (Weed, 1970; Mohandas et al, 1979). During storage in the liquid state red cells progressively lose their viability (Card et al 1982); this loss is associated with loss of membrane lipid (Haradin 1969) and with reduced deformability (Card et al, 1983). Deformability of red cell membranes can be measured using osmotic gradient ektacytometry. Characteristic profiles are obtained and can be used to detect changes in membrane surface area and water content.

Clark et al (1983), have shown that by selectively altering the membrane area and water content of normal cells three features of the osmotic deformability profile can be used to characterise cell populations:

(i) The osmolality at which hypotonic minimum deformability or elongation (E1 min) occurs, designated 0 min, provides a measure of the average ratio of surface area to volume of the cell population. This value coincides with that of the osmolality at which 50% of the cells haemolyze in an osmotic fragility test.

(ii) For homogenous cell populations with normal or reduced lipid content, the height of the maximum deformability or elongation index (E1 max) reflects the amount of membrane surface area available for cell deformation. The position of E1 max moves to lower or higher osmolality (0 max) as the water content of the cell is decreased or increased, whereas a reduction in membrane surface area causes a reduction in maximum attainable E1.

(iii) A measure of initial water content of the cell can be derived from the hypertonic area of the profile by determining the osmolality at which E1 reaches a particular value, since cell deformation is also determined by intracellular viscosity.

In order to try and elucidate the mechanism by which DEHP extracted from PVC blood bags binds to the red cell membrane and stabilises it, Labow et al (1987) measured the red cell deformability using the osmotic gradient ektacytometer and correlated this to the DEHP concentration during storage, relating the characteristic measurements (0 min, E1 max, 0 max) to the osmotic fragility and adenosine triphosphate (ATP) content measured on the same samples.

Pooled red cell concentrates (RCC) were aliquoted into DEHP plasticised PVC bags (PL146) polyolefin bags (PL732) and polyolefin bags with added DEHP. The PL732 and PL146 containers are manufactured by Fenwal Division, Baxter Health Care, Round Lake, Illinois, USA and find extensive use in red cell and platelet storage (see Chapter 7) respectively. Dimethyl sulphoxide (DMSO) was used to dissolve the DEHP to give a final concentration of 150μg/ml RCC, and the same amount of DMSO was added to the second polyolefin (PO) container.

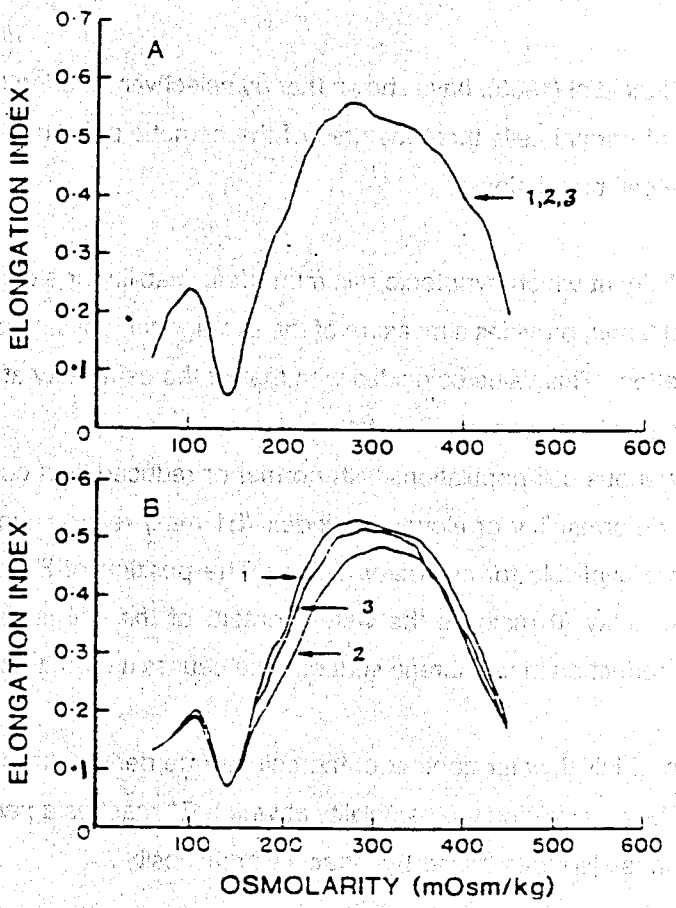


Fig 6.4 RC deformability measured in RCC stored in three types of bags: PL146, PL732 plus DMSO, and P)L732 plus DMSO and DEHP. (A) Day O; (B) day 35.

(Labow et al, 1987)

Samples were removed at weekly intervals from the 3 containers for analysis of osmotic fragility, deformability and DEHP concentration as shown in Table 6.9 below.

Table. 6.9 DEHP concentration (μg/ml RCC)

DAY	PVC	PO plus DMSO	PO plus DEHP in DMSO
0	22 ± 1.1	2.7 ± 1.4	155 ± 57
7	54 ± 19	4.2 ± 1.4	95 ± 10
14	89 ± 34	6.0 ± 2.1	78 ± 3
21	116 ± 54	7.4 ± 3.2	85 ± 11
28	141 ± 77	8.4 ± 4.0	78 ± 17
35	174 ± 88	9.2 ± 4.5	66 ± 12

The adenosine triphosphate (ATP) concentration in the RCC decreased with increased storage time to the same extent in all three types of storage conditions, with minor differences observed which were not statistically significant. This effect showed that DEHP exerted its effect directly on membrane flexibility and not on red cell function.

The DEHP concentrations in each of the storage containers changed as predicted. The variation in the DEHP concentration (table 6.9) is due to variation in the amount by weight of pooled RCC in the PVC storage container. In the standard PVC container normally used for RCC storage, DEHP continued to be extracted increasing to 178 ± 88μg/ml by day 35. When DEHP is added to the PO container at 0 time, there is a drop in concentration with time due to the conversion of DEHP to MEHP (Rock et al, 1978).

The PO container, although manufactured without DEHP, appeared to contain small quantities of this plasticiser as small amounts (<10μg/ml) were extracted during the storage period. No explanation was given regarding its presence, although the difference in DEHP concentration in the PVC and PO containers is so great that it is evident that the results obtained when measuring red cell deformability and osmotic fragility were directly related to the DEHP concentration measured in the RCC.

Figure 6.4 shows the red cell deformability for red cell storage under all three storage conditions. There was a statistically significant difference between PL732 plus DMSO alone compared with PL146 or PL732 plus DMSO and DEHP, clearly seen when E1 max, 0 max and 0 min values are plotted separately (fig 6.5). The 0 min values correlated directly with the 50% haemolysis point on the osmotic fragility curves (fig 6.6). There was no statistically significant difference between the PVC or the PO containers in the 50% haemolysis point up to day 21 of storage. After day 21, it was evident that the increased concentration of DEHP caused a decrease in the osmotic fragility. The shift to higher percentage sodium chloride was equal to the 0 min shift on the deformability profiles on day 35 of storage.

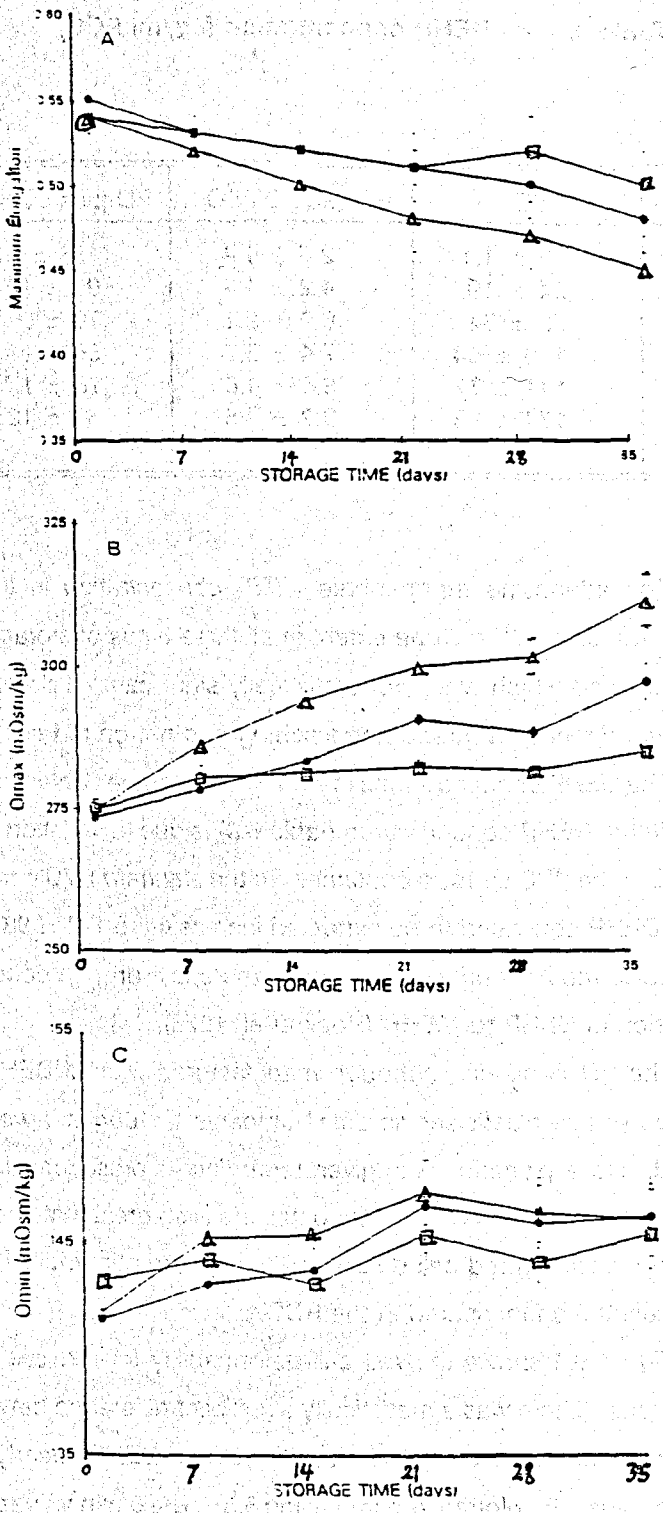


Fig 6.5 The variation of El max (A), Omax (B), and Omin (C) with increasing storage time in three types of storage containers:
PL146, △ PL732 plus DMSO, ▪: and PL732 plus DMSO and DEHP, ●.

(Labow et al, 1987)

In addition there was a decrease in the maximum elongation index (E1 max) when there was decreased DEHP in the storage bag. The osmolarity (0 max) at which E1 max occurred, as well as the 0 min, the osmolarity at which minimum elongation (E1 min) occurred was higher in the PO (PL732) container than in the DEHP plasticised PVC container (PL146) or the PO container plus added DEHP. These charges could be reversed by addition of DEHP at the beginning of the storage period, showing a direct correlation between DEHP concentration during storage and red cell membrane flexibility (Labow et al, 1987).

6.5 THE EFFECT OF DEHP ON THE SURVIVAL OF STORED RED BLOOD CELLS

The in vitro studies reported in the previous sections of this chapter have demonstrated that the storage of RBC's in the presence of DEHP results in a better retention of normal morphology enhanced osmotic stability, and lower haemolysis than is observed during storage in the absence of this plasticiser. However in none of these studies was the effect of DEHP on in vivo RBC survival investigated. The experiments reported by AuBuchon et al (1988) were performed to ascertain whether the previous in vitro observations were indicative of a clinically relevant improvement in RBC function. Whole blood collected from ten normal donors were stored for 35 days in citrate-phosphate-dextrose-adenine (CPDA-1) anticoagulant contained in PVC blood bags plasticised with DEHP or trimellitate (TOTM). Aliquots of RBC's from each container were then labelled with chromium-51 and were reinfused into the original donors. The experimental protocol used is illustrated in table 6.10.

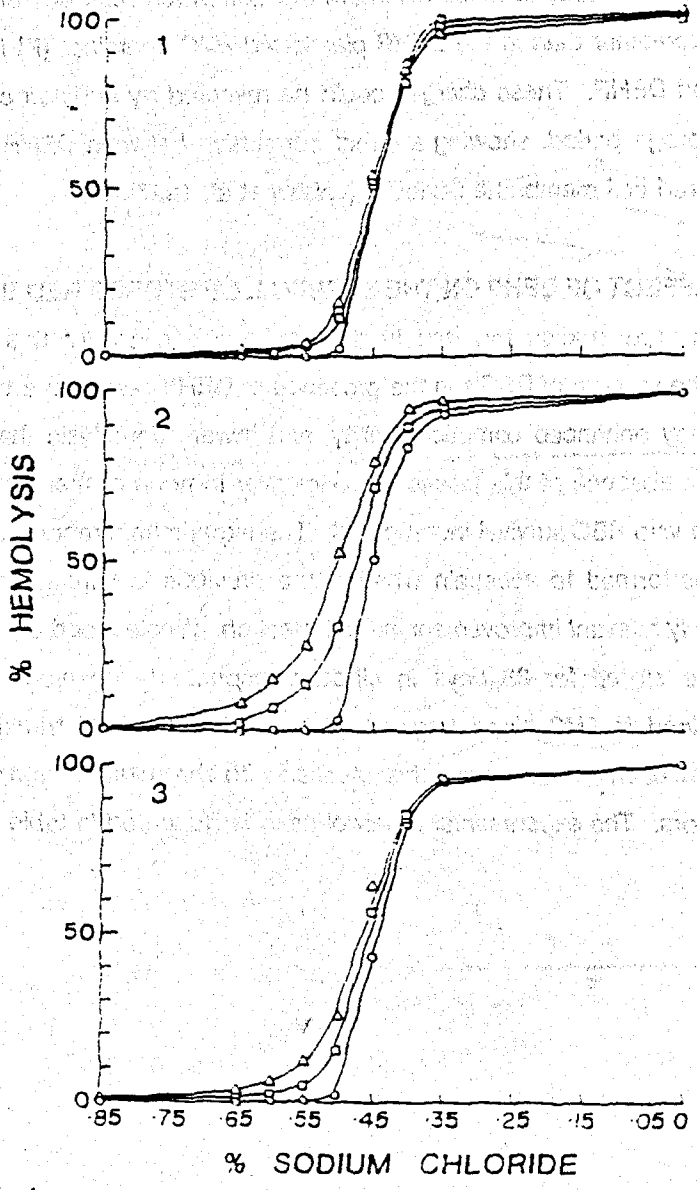


Fig 6.6 ***The variation of osmotic fragility with increasing storage time in three types of containers: PL146, PL732 plus DMSO, and PL732 plus DMSO and DEHP, Day 0: O: day 21 □ ; and day 35, △***

(Labow et al, 1987)

Table 6.10 Experimental Protocol

Phase	Component Stored	Group	Container*	Additions	Subjects Studied
i	Whole blood	A[1]	PVC-TEHTM[2]	None	10
		B	PVC-DEHP[3]	None	
ii	Whole blood	C[1]	Glass[4]	None	5[5]
		D	Glass[4]	DEHP[6]	
iii	Packed red cells	E[1]	PVC-TEHTM	None	8
	(Hematocrit:75%)	F	PVC-DEHP	None	

* All blood collected into CPDA- 1 anticoagulant and stored for 35 days. Blood collected directly into final storage container. Approximately 60 mL of blood removed on days 0 and 35 for analytic determinations.

1 Blood drawn from donor using an aluminium-hubbed needle and polyethylene tubing. Standard blood-drawing sets used for other collections.

2 PVC bag plasticized with tri-2 ethylhexyltrimellitate, a plasticizer of low leachability and red cell effect (PL 1240; Fenwal, Deerfield, IL).

3 PVC bag plasticized with di-2-ethylhexyl phthalate (PL 146; Fenwal).

4 Sterile, vented 1-L glass bottles (Travenol, Deerfield, IL). Bacterial cultures performed on day 28.

5 These subjects also participated in phase 1. Two system D units became bacterially contaminated and were not used.

6 In group D samples. 20 mL of plasma were harvested (1.100 g for five minutes) form the day 0 aliquot, removed as stared above. This was frozen in sterile tubes in 4 mL aliquots. On days 0, 7, 14, 21, and 28, 0.8 mL DEHP(99 + % purity; Eastman, Rochester, NY) was added to one of these plasma aliquots and incubated at 37°C fr two hours with periodic agitation. The plasma was then centrifuged (500 g for five minutes), and 2 mL of plasma were added aseptically to the storage container with gentle agitation. **(AuBuchon et al, 1988).**

Table 6.11. Effect of DEHP on post-transfusion survival of red cells

Storage System	Container Type	Subjects Studied	24-h Survival (%)	T^{50} (0-10 min)
Phase I				
A	PVC-TEHTM (PL 1240)		69.9 ± 2.99	32.4 ± 5.16
		10		
B	PVC-DEHP (PL 146)		84.1 ± 2.13	76.8 ± 12.4
Phase II				
C	Glass, No DEHP		73.3 ± 2.69	20.5 ± 2.50
		3		
D	Glass + DEHP		81.7 ± 2.23	22.7 ± 4.56
Phase III				
E	PVC-TEHTM (PL 1240)		66.5 ± 3.29	42.7 ± 8.32
		8		
F	PVC-DEHP (PL 146)		75.8 ± 1.87	61.1 ± 16.1

T^{50} (0-10)is the time taken for survival to fall to 50% of original value according to regression line for survival curve during first ten minutes after reinfusion

Pairs statistically different by paired *t* test, P, .001.

Pairs statistically different by paired *t* test, P, .05

(AuBuchon et al, 1988)

RBCs stored in systems containing DEHP exhibited improved survival rates after transfusion than did red cells stored in similar containers that lacked DEHP as illustrated in table 6.11. After storage for 35 days as whole blood, red cells stored in PVC bags with DEHP (System B) exhibited a 24-hour post-transfusion survival of 84.1% ± 2.13%, 17% higher than that of red cells stored as whole blood in PVC bags lacking DEHP (System A: 69.9% ± 2.99%, P <.001). Only three pairs of data from phase II could be analysed because of inadvertent bacterial contamination of two system D units. In spite of the small sample, storage of whole blood in glass bottles to which DEHP was added (System D) resulted in a 24-hour red cell survival, 81.7% ± 2.23%, that was statistically significantly higher than storage in System C, which lacked DEHP (73.3% ± 2.69%, P<.05). The survival of red cells stored as packed red cells was lower than that of cells stored as whole blood. In phase III the presence of DEHP in the storage system was again associated with improvement in post-transfusion survival. Red cells stored in a PVC bag with DEHP (System F) had a post-transfusion survival of 75.8% ± 1.87%, 14% higher than that of cells stored in a PVC bag without DEHP (System E: 66.5% ± 3.29%, P<.05).

The difference in survival of red cells after storage was quantitatively attributable to the altered rate of disappearance of red cells from circulation during the first ten minutes after infusion (fig 6.7). For example, in Phase I, the ^{51}Cr T_{50} for this period was 32.4 ± 5.16 minutes for red cells stored in PVC plasticised without DEHP (System A) and 76.8 ± 12.4 minutes for the PVC-DEHP system (System B; P <.001). The rate of red cell disappearance from circulation from ten minutes onward was not significantly different between storage with and without DEHP (fig 6.7).

In agreement with previously reported studies, DEHP accumulated in units of blood stored in PVC bags that used this compound as a plasticiser. During 35 days of storage in System B, the whole blood concentration of DEHP had risen from undetectable levels to 146 ± 12.6 μg/ml. By performing a least-squares linear interpolation of the data derived from the units stored in System B and nine therapeutic phlebotomy units, the accumulation of DEHP in PL146 whole blood units could be expressed as:

$$24.0 + 3.29\mu g/ml \times (\text{days of storage})$$

$$(n = 49,\ r = 0.8010,\ P < .0001)$$

DEHP was successfully solubilised in autologous plasma for addition to glass bottles in system D. The five additions added 49.0 ± 9.6 mg DEHP to the system. The concentration of DEHP rose throughout storage and was 19.0 ± 0.60μg/ml on day 35.

Red cells from phase III systems were tested for the presence of C3 on their surface by a direct antiglobulin test using anti-C3 after 35 days of storage. All samples from systems E and F were negative.

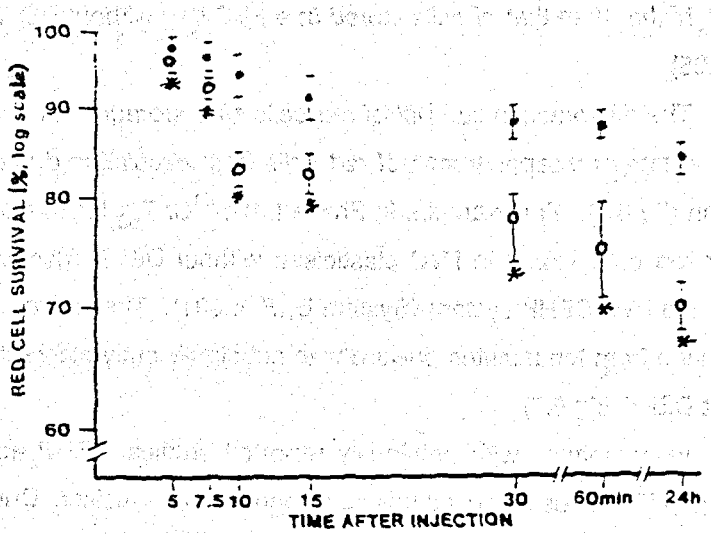


Fig 6.7 Red cell survival after storage for 35 days as whole blood in PVC bags with (●) or without (O) DEHP. Survivals were compared in ten donors after storage in each container type. Note that difference in survival at 24 hours is quantitative apparent within the first ten minutes after reinfusion. Vertical bars represent SEM; asterisks indicate statistical significance, P<.05.

(Aubuchon et al, 1988)

The paired in vitro study of red cells stored in PVC with or without DEHP and then subjected to radiolabelling and in vitro elution estimation demonstrated similar uptake and elution of the ^{51}Cr label regardless of the plastic formulation used to store the red cells (table 6.12).

Table 6.12 Effect of DEHP on Red Cell Uptake and Elution of ^{51} Cr in Vitro

Storage	Labelling	Proportion of Radiolabel Eluting (%)			
Container	Efficiency	0 min	10 min	60 min	24 h
PVC-TEHTM (PL 1240)	96.9% ± 0.36	0.28 ± 0.052	0.52 ± 0.20	0.54 ± 0.068	1.7 ± 0.13
PVC-DEHP (PL 146)	96.4% ± 0.51%	0.33 ± 0.057	0.39 ± 0.066	0.61 ± 0.087	1.9 ± 0.19

Whole blood from 11 single donations was split between PVC bags lacking (PL 1240) or containing (PL 146) DEHP and stored for 35 days in CPDA-1. After ^{51} Cr labelling, red cells were held in AB plasma at 37°C, and plasma radioactivity was determined as a percentage of the radioactivity originally taken up by the red cells. There were no statistically significant differences in the uptake or loss of the radiolabel between red cells stored in the two bag types.

In agreement with previous studies Labow et al (1987), the presence of DEHP did not alter the disappearance of ATP from red cells during the storage period. It did however mitigate the normal increase in plasma haemoglobin accumulation as shown in table 6.13 overleaf.

Table 6.13 Effect of DEHP on Red Cell Metabolism and Integrity

Storage System	Container Tube	ATP Concentration (% Day 0 Concentration)	Haemoglobin Concentration (mg/dL) Plasma	Ultracentrifuged Plasma	Microvesicle Fraction
Phase I					
A	PVC-TEHTM (pl 1240)	53.9 ± 7.08	178±40.8	102±33.0	76.4±15.6
B	PVC-DEHP(PL 146)	62.1±9.51	73.1±17.4*	37.0±12.4*	37.1±9.12
Phase II					
C	Glass, No DEHP	57.1±10.7	ND	ND	ND
D	Glass + DEHP	61.2±12.9	ND	ND	ND
Phase III (packed red cells)					
E	PVC-TEHTM(PL 1240)	58.9±7.01	915±257	801±221	167±91.8
F	PVC-DEHP(PL 146)	57.5±5.65	350±97.8*	231±59.9*	125±43.7

Determinations performed before and after 35 days of storage in CPDA-1. Haemoglobin concentrations corrected for plasma haemoglobin concentration on day 0. Difference between plasma and ultracentrifuged plasma on day 35 taken as haemoglobin in microvesicle fraction.

Abbreviation: ND, not done.

* Pairs statistically different by Mann-Whitney rank sum test. $P<.05$.

(Horowitz et al, 1985)

The relative increase in plasma haemoglobin concentration in blood stored without DEHP was accompanied by an increase in microvesicle content, but the latter could not quantitatively account for the total increment of plasma haemoglobin concentration.

The studies by AuBuchon et al (1988) demonstrated that the post-transfusion survival of RBCs stored under refrigeration for 35 days in CPDA-1 anticoagulant in DEHP plasticised PVC containers was on average 17% to 24% higher than the survival of cells stored in containers plasticised with TOTM. Enhanced survival was also observed when DEHP was added to whole blood stored in glass bottles, providing further evidence that the improved post-transfusion survival was attributable to the presence of the plasticiser. As demonstrated by other in vitro studies, the presence of DEHP in clinically relevant quantities does not directly affect the internal metabolism of red cells (Stern & Carmen, 1980; Estep et al, 1984; Rock et al, 1984). Furthermore as with previous studies, ATP levels, 2.3-DPG levels and intracellular electrolyte concentrations are not affected by the presence of DEHP (Estep et al, 1984).

While other mechanisms cannot be completely excluded, the enhanced post-transfusion survival of red cells stored in the presence of DEHP reflects its protective effect with regard to the deterioration of the red cell membrane. Previous in vitro studies reported in this chapter illustrated that RBCs stored in the presence of DEHP exhibit more normal morphology, less haemolysis, lower osmotic fragility and better filterability than do comparable cells stored in its absence. Each of these parameters is believed to reflect the integrity of the cell membrane. Cell morphology, in particular, has been closely correlated with in vivo cell survival (Hogman et al, 1980; Hogman et al, 1985).

The RBC storage in the presence of DEHP as assessed by in vitro measurements is therefore indicative of an improvement in in vivo cell survival as described.

CHAPTER 7

CHAPTER 7.

PLATELET CONTAINERS, SELECTION OF POLYMERIC MATERIALS

7.1 INTRODUCTION

The therapeutic value of transfused platelets was first demonstrated by Duke (1911). In three thrombocytopenia patients, two of whom were children, bleeding stopped after the direct transfusion of fresh blood; in all cases, the bleeding time diminished and in the two cases in which platelet counts were done, an increase was observed lasting about 3 days. Despite this early recognition of the potential value of platelet transfusion there were many subsequent reports of poor results. Some at least were explained when it was shown that, in patients with idiopathic (autoimmune) thrombocytopenia purpura, transfused platelet are rapidly destroyed. Others were doubtless due to technical difficulties. With modern technology, platelet concentrates can be prepared which are often of great benefit to severely thrombocytopenia patients (Mollison et al, 1987).

In chapter 2 the necessity for flexible polymeric containers for the collection of platelet and the preparation and storage of platelet concentrates was described. This chapter considers the available choice and design of polymeric materials and factors influencing their selection.

7.2 CONVENTIONAL DEHP PLASTICISED PVC PLATELET CONTAINERS

As stated in chpater 2, the poor survival of platelets stored in whole blood and the need to provide platelet preparations rapidly from whole blood which has been stored for less than 24 hours has already been discussed. With the introduction of a system of flexible PVC packs with integral tubing for collection, processing and storage, together with the parallel development of high speed refrigerated centrifugation the means became available for the preparation of sterile blood components. Initially and until quite recently there was no differentiation between the use of DEHP plasticised PVC for the primary blood bag interconnecting tubing and its satellite containers. Under these conditions, the storage period for platelet concentrates (PCs) was limited to 3 days (Slichter & Harker, 1976; Murphy, 1985; Heaton, 1986).

During storage, the pH decreases, and it has been demonstrated that below pH 6.1 there is a severely compromised survival of transfused platelets (Murphy 1970, Becker 1973). The decrease in pH is accompanied by an increase in plasma lactate and Pco_2 (Hardeman 1975, Rock 1981). Under these conditions, platelet viability is only 40 percent after 72 hours of storage (Slichter & Harker 1976). Considerable data exist showing a significant relationship between the decrease in pH and the increase in CO_2 of the plasma, and this has pointed to the importance of carbon dioxide removal in effecting changes during storage (Murphy 1975, Rock 1976).

When platelets are stored in conventional DEHP plasticised PVC platelet bags,

the plasticiser migrates into the plasma and is bound to the platelet membrane; however, unlike the situation with red cells where the DEHP concentration was 50 percent in the cytosol and 50 percent in the membrane (Rock et al 1984), the compartmentalisation in the platelet was 5:95, cytosol : membrane (Labow et al, 1986). High levels of DEHP have been demonstrated in platelet concentrates (PCs) during storage in PVC bags Jaeger & Rubin (1973), such as the PL146 platelet container supplied by Baxter Fenwall Laboratories, Deerfied, Illinois. As it accumulates during storage, DEHP is converted to mono(2-ethylhexyl) phthalate (MEHP) by an enzyme located in plasma (Rock et al, 1978). The accumulation of MEHP in platelet preparations is of concern as already referred to earlier, and has been shown to be 20 times more toxic than DEHP in rats (Chu et al, 1978).

In the early 1980's, the major blood bag manufacturers began to address these questions with the introduction of alternative polymeric formulations specifically designed for platelet collection and storage (table 7.1), the characteristics of which permit increased gas permeability and prolonged storage of platelet concentrates. A number of in vitro and in vivo studies have been performed to examine the effect of extended storage in these containers (Gunson et al, 1983; Simon et al, 1983; Murphy et al, 1984; Rock et al, 1984; Labow et al, 1986).

Table 7.1 Platelet storage containers

PLATELET CONTAINER	MANUFACTURER	POLYMER TYPE	PLASTICISER	SIZE (ml)
PL 146	BAXTER FENWALL	PVC	DEHP	300
CL 3000	CUTTER LABORATORIES	PVC	DEHP	300
PL 1240	BAXTER FENWALL	PVC	TOTM	300
CLX 300	CUTTER LABORATORIES	PVC	TOTM	300
F 702	BIOTEST PHARMA	PVC	TET	300
PL 732	BAXTER FENWALL	POLYOLEFIN	NONE	300, 1000

7.3 TOTM PLASTICISED PVC PLATELET CONTAINERS

Extension of the storage time of platelet concentrates in a satellite bag which employed TOTM plasticised PVC was studied by reinfusing autologous ^{51} Cr-labeled platelets into normal volunteers and measuring postinfusion platelet counts and bleeding times in patients requiring platelet transfusions (Simon et al, 1983). The use of the new storage medium was found to protect platelet concentrates against fall of pH when compared with two conventional DEHP plasticised PVC platelet containers (PL146 and CL3000) included in this study. This protection was felt to be due to the greater gas permeability of the new plastic CLX system as illustrated (table 7.2).

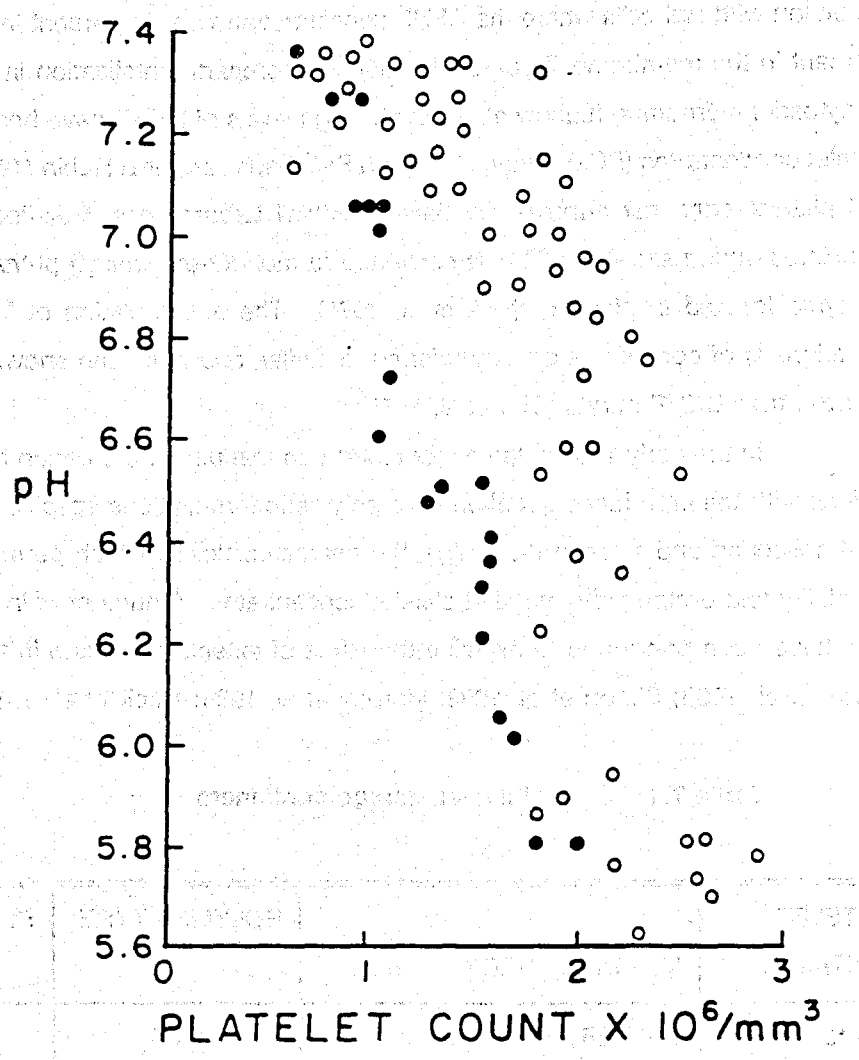


Fig 7.1. **The relationship of platelet count in PC and poststorage pH. ● = platelet concentrates stored in PL-146 for 3 days; O = platelet concentrates stored in the satellite bag from the CLX™ system for 5 days. pH maintenance is better when the platelet count is in the range 1-2 x 10^6 per μl.**

(Simon et al, 1983)

Table 7.2 Gas transmission rate, ($cc/m^2/day$) across the study films

	Oxygen	Carbon Dioxide
CLX™ system		
Test 1	690	4100
Test 2	690	3830
PL-146		
Test 1	445	1180
Test 2	490	1375
CL-3000		
Test 1	465	1585
Test 2	470	1890

(Simon et al, 1983)

The plastic film from the CLX system was more permeable to oxygen and carbon dioxide than other commercial PVC blood bag films (table 7.2). TOTM plasticiser levels in blood components prepared and stored in CLX bags were lower than DEHP levels from other commercial bags when platelet concentrates were stored at 22°C and subjected to both horizontal and end-over-end agitation. Laboratory studies performed on platelet concentrates with counts in the range of a 1 to 2 x 10^6 per μl (equivalent to 5-10x10^{10} platelet in 50 ml of plasma) showed that the poststorage pH/platelet count relationship for 5 day bags from the TOTM system (CLX bags) was satisfactory in the majority of concentrates (fig 7.1). A pH fall to less than 6.0 was seen after 5 days of storage in the TOTM satellite bags with platelet counts of 2 x $10^6/\mu$l or greater (equivalent to 10 x 10^{10} platelets in 50ml of plasma). Data obtained in the course of the patient studies showed that pH fall below 6.0 occurred in only 10.8 percent (18 out of 167) of routinely prepared concentrates stored for 5 days. Such pH fall was seen in 5 of 20 units stored for 7 days (Simon et al, 1983).

Mean in vivo recovery and half life (greater than 31% and 3.3 days respectively) of autologous reinfused platelet were satisfactory following 5 days of storage. Following 7 days of storage, mean recovery was 41 per cent and half-life was 2.8 days. Peripheral platelet count increments in patients following platelet transfusions with concentrates stored 4 to 7 days in TOTM plasticised PVC bags were comparable to increments following transfusion of platelet stored 2 to 3 days in DEHP plasticised PVC containers. Bleeding times shortened in three of four patients receiving platelet concentrates stored form 4 to 6 days in the new system. Platelet concentrates stored in the CLX containers at 20 to 24°C with flat-bed or elliptical agitation could be transfused up to 5 days following phlebotomy with acceptable clinical results.

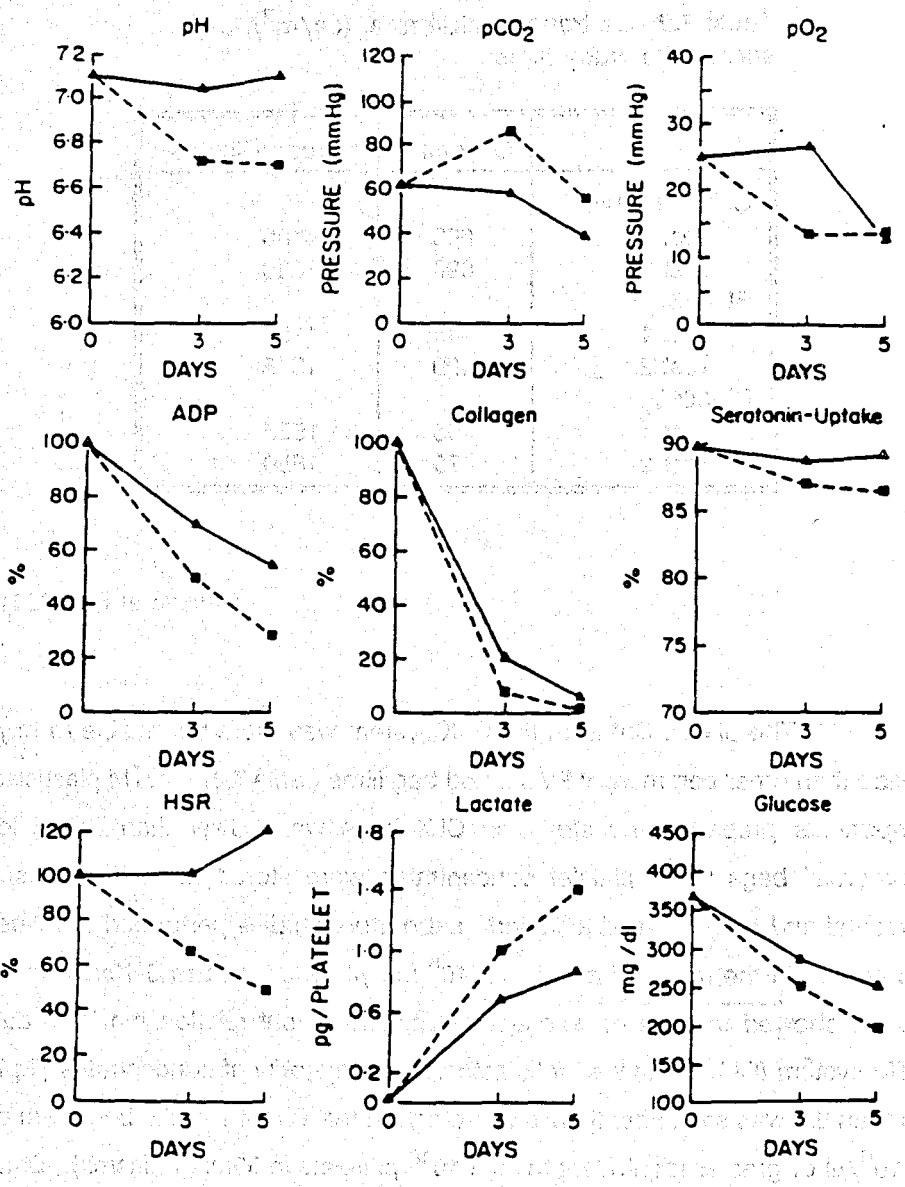


Fig 7.2. A comparison of platelet function after storage in CLX or PL146. Pooled platelet concentrates were studied during storage for 5 days in 300-ml, PL146 and CLX plastic bags. CLX, n = 12; PL146, n = 6, platelet count, 900 - 1200 x 10^9 per liter. ■—■ , PL146; ▲—▲ , CLX.

(Rock et al, 1984)

7.4 POLYOLEFIN PLATELET CONTAINERS - A COMPARISON WITH DEHP AND TOTM PLASTICISED SYSTEMS

An alternative non PVC platelet container has been developed by Baxter Fenwell in the form of a polyolefin (PL732, see table 7.1). The polyolefin (PO) is stated by the manufacturer to be essentially free of liquid plasticiser, although it does contain an anti-oxidant 1,3,5-trimethyl-2,4,6-tris (3,5-di-tert-butyl-4-hydroxybenzyl) benzene (BHBB). It is interesting to note that the use of antioxidants is not permitted in flexible PVC formulations destined for blood contact as described in European Pharmacopoeia Monograph V1.1.2.1.1 (see fig 3.5). Storage of platelet concentrates has been extended to 5 days in the PL732 system according to Murphy et al, (1982). Rock and co-workers, (1984) examined platelet stored in both PL732 Baxter Fenwall and CLX Cutter Laboratories systems (referred to in the previous section) and have assessed both in vitro and in vivo function after 5 days of storage. They also undertook a direct comparison between these two bags after storage for 7 days and an evaluation of different methods of agitation.

The blood bags used in this study were obtained from the manufacturers. The primary pack for the PL732 bag was composed of PL146 (DEHP plasticised PVC) whereas all bags of the CLX system where based on TOTM plasticised PVC. No mention is made of the composition of the associated tubings used in these devices. Due to the rapid rate of extraction by whole blood of DEHP from PL146 or similarly plasticised PVC containers the presence of DEHP in platelet preparations stored in PO containers cannot be excluded. In the case of the CLX system the application of TOTM plasticised flexible PVC tubings, as increasingly used for haemodialysis tubing, Flaminio et al (1988), Christensson et al (1991), would avoid any potential cross contamination by DEHP. This point however remained unclarified. All primary bags contained CPDA-1 anticoagulant. Platelet were counted and sized using a Coulter Counter with a 50μ aperture. Platelet aggregation was performed on a lumi-aggregometer (Chrono-Log Corporation) using ADP (final volume 10μM), epinephrine (50μM), collagen (40μg/ml), and ristocetin (1.2 mg/ml). In addition hypotonic shock response (HSR) was studied (Odink, 1974). Serotonin uptake and release following simulation with ADP were calculated. Measurements of Pco_2, Po_2, and pH were made on a blood gas analyser, while lactate accumulation and glucose content of the plasma were assessed by means of biochemical kits. Evaluation of gas escape was performed by volume displacement and release of $^{14}C\text{-}CO_2$ (Rock et al 1981).

The results recorded for CO_2 and O_2 gas escape are shown in Table 7.3. The comparison of platelet function after storage in TOTM plasticised (CLX) and DEHP plasticised (PL146) PVC containers appear as fig 7.2; and that between the PO (PL732) and DEHP plasticised (PL146) PVC container is shown in figure 7.3.

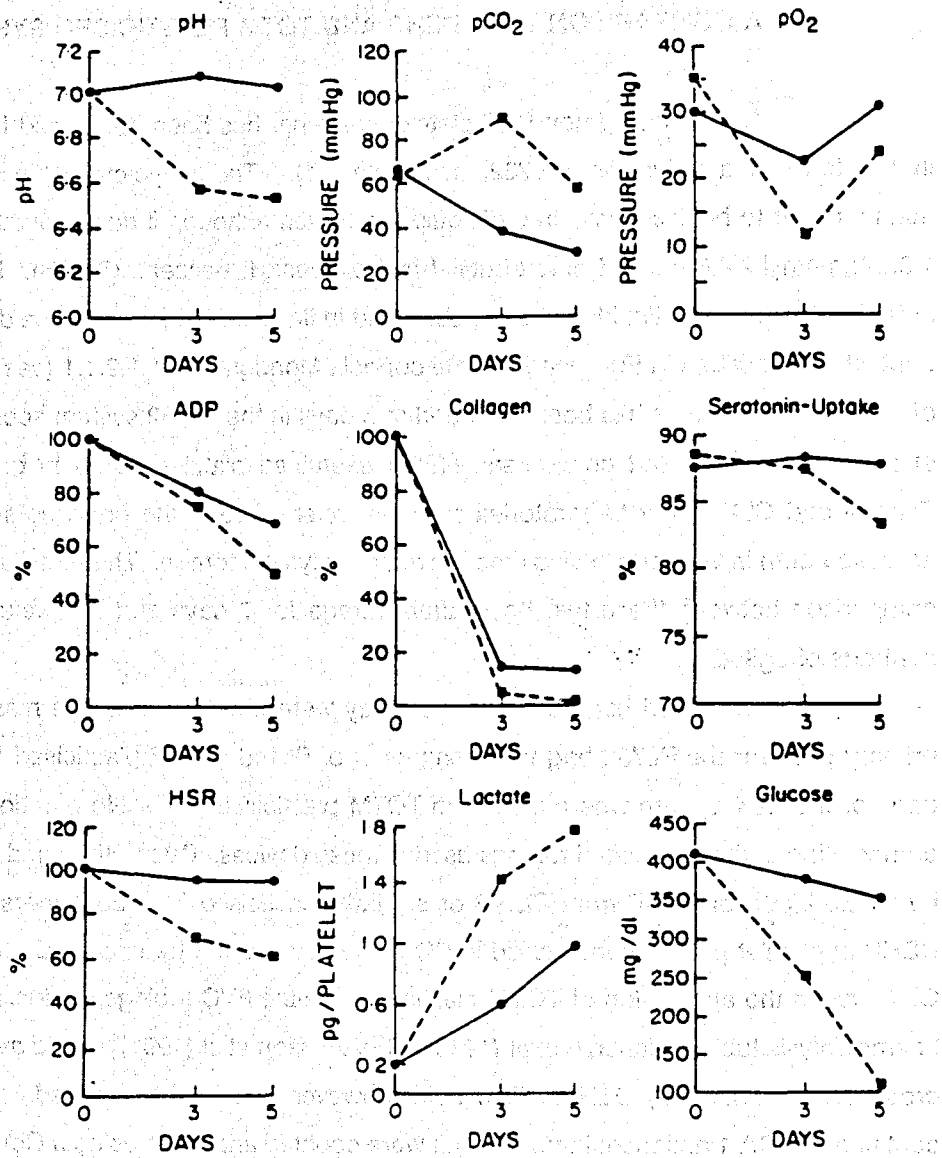


Fig 7.3 **Platelet function after storage in PL732 or PL146. Pooled platelet concentrates were assessed during storage for 5 days in 300-ml, PL732 and PL146 bags. PL732. n = 12; PL146.n = 4; platelet count, 900 - 1400 x 10^9 per liter. ▪—▪. PL146; ● _____ ●, CLX.**

(Rock et al, 1984)

7.4.1 Comparison of TOTM and DEHP plasticised PVC platelet bags (Rock et al, 1984)

When pools of platelet concentrates were split and stored in either the normal DEHP plasticised or the new TOTM plasticised bags the platelet had better in vitro function in the latter (fig 7.2). Using reciprocating flat-bed agitation, platelets stored for 5 days in the CLX maintained the pH between 7.0 and 7.5. In contrast, the pH values of concentrates stored in the PL146 bags decreased by 3 days and after 5 days were below acceptable limits in many cases. Less lactate accumulated and less glucose was consumed in the CLX bag. Aggregation responses to ADP, collagen and epinehrine were the same or better in the CLX bag and the HSR was maintained well, whereas it progressively decreased int the PL146. The Pco_2 decreased over time in the CLX bag; in contrast, there was an increase in Pco_2 in the standard bag by 3 days. Oxygen levels decreased during storage in both bags but to a greater extent in the standard bag. This difference in escape of CO_2 and O_2 was substantiated by the data for the relative permeability of the plastic: the ^{14}C-CO_2 measurements showed 30 per cent removal of the carbon dioxide in the CLX bag by day 5 and only 26 per cent removal in the PL146 (table 7.3). Oxygen loss, as measured by volume displacement, was slower with 14% less form both the CLX and the PL146 after 3 days.

Table 7.3 Measurement of CO_2 and O_2 gas escape from plastic bags

	CLX		PL732		PL146	
	Day 3	Day 5	Day 3	Day 5	Day 3	Day 5
pH	-	8.0	-	8.3	-	7.8
^{14}C-CO_2*	25.5	30.4	29.8	36.9	18.6	26.1
CO_2**	95	97	93	93	84	93
O_2**	14	17	31	34	14	17

* Per cent gas loss from platelet-poor plasma (PPP) in the different plastic bags.

** Percent gas loss by volume displacements.

(Rock et al, 1984)

^{51} Cr survivals were performed on four normal donors who gave informed consent for infusion of autologous platelet stored for 5 days in either PL732 or CLX bags or for 3 days in the PL146 bag. ^{51}Cr survival of autologous platelets stored for 5 days in CLX bags was 5.1 ± 1.4 days. The postinfusion recovery of the radio label was 58 per cent at 2 hours. Control survival values (Rock et al, 1984) are 5.4 ± 1.0 days for 3 day old platelets stored in PL146 bags. Following infusion into six thrombocytopenic patients, the 5 day-old platelets showed 49 per cent recovery after 1 hour and 31 per cent recovery at 24 hours.

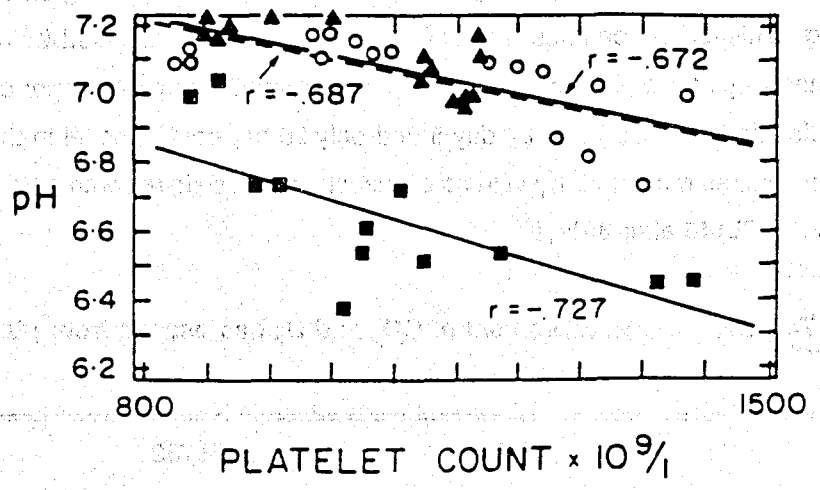


Fig 7.4 The relationship of pH and platelet count following storage in PL146, PL732, and CLX bags. Pools of platelet concentrates were stored for a) 3 days in Pl146. b) 5 days in PL732. and c) 5 days in CLX. Linear least-squares regression analysis was used to determine correlation coefficient (r) ▪ ___PL146: ___CLX: O___PL732.

(Rock et al, 1984)

7.4.2 Comparison of PO and DEHP plasticised PVC platelet bags (Rock et al, 1984)

The PL732 platelet bag also allowed better maintenance of in vitro platelet function than the PL146 bag. After 5 days of storage, the pH showed little change and the lactate accumulation was lower than that typically found after 3 days of storage in PL146 containers (fig 7.3). Little glucose was consumed over the 5 days, and serotonin uptake and HSR showed better maintainance. Increased permeability was reflected in the lower Pco_2 and higher Po_2 at 5 days. The measurement of $^{14}C\text{-}CO_2$ escape showed 37 per cent removal at 5 days: 34 percent of the oxygen had escaped at this time (table 7.3). Autologous infusion of platelets stored for 5 days in the PL732 bags showed a survival time of 6.0 ± 2.8 days with 40 per cent recovery at 2 hours. Post transfusion increments in thrombocytopenic patients were good with 9.9×10^9 per 1 per 10^{11} platelets transfused per m^2 with 44 per cent recovery at 1 hour and 2.8 per cent at 24 hours.

The significance of the PO and TOTM plasticised PVC platelet bags in maintaining pH is shown in fig 7.4, where for the same platelet count, less change shown in comparison with PL146 and mirrors the findings of Simon et al (1983) see fig 7.1 when comparing PL146 and CLX containers. The effect on platelets after 5 and 7 days storage in both PO and TOTM plasticised platelet bags are demonstrated (fig 7.5). In general, while some differences could be shown, there were relatively few benefits to be derived by using one bag over the other. The pH was maintained above 6.9 for both bags even after 7 days of storage, and there was relatively little difference in aggregation response. The HSR was slightly better in the TOTM plasticised PVC bag, but serotonin release and uptake were similar. The relative rate of lactate accumulation was also very similar, showing a steady increase with time from day 0 to day 7. While CO_2 was lost at a similar rate, the diffusion of oxygen into the plasma was higher for the PO bag at 5 and 7 days than for the TOTM plasticised PVC container.

7.5 THE INFLUENCE OF POLYMER SELECTION ON PLATELET MORPHOLOGY AND FUNCTION

Fratantoni et al (1984) have observed unusual morphological alteration in platelets sorted in both TOTM plasticised PVC (CLX) and polyolefin (PL-732) platelet containers after 2 or 3 days of storage. The atypical forms observed included crescents, elongated tubular forms and rings. No such pattern of distribution has been reported when platelets are sorted in DEHP plasticised PVC containers, although atypical forms can be found in small numbers when such platelet concentrates are carefully examined.

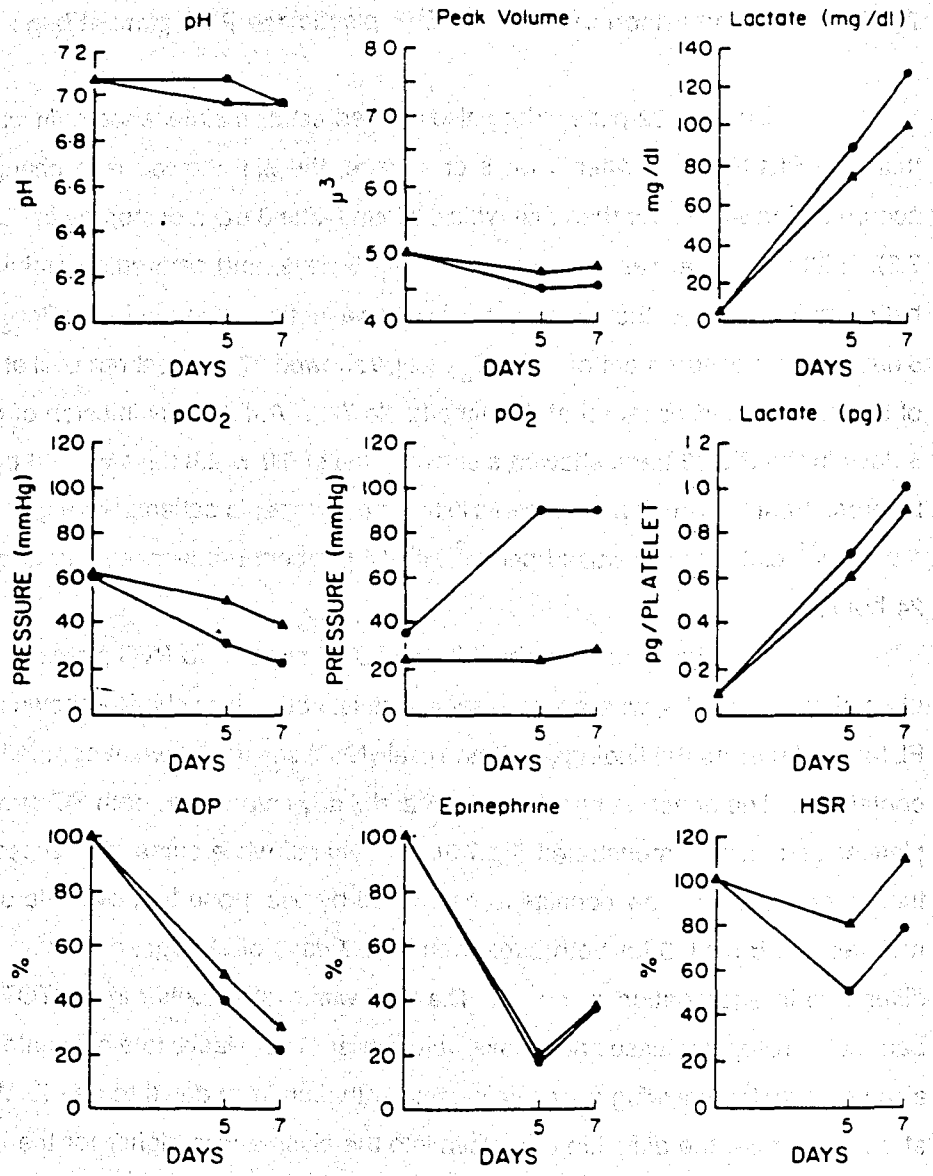


Fig 7.5 **A comparison of in vitro function of platelet concentrates stored 7 days in CLX and PL732 bags. The platelet concentrates were stored on a reciprocating flat-bed agitator for 7 days. Platelet function variables were assessed after 0, 5, and 7 days of storage at 22°C. N = 3; platelet count, 1100 x 10^9 per liter. ▲——▲. CLX; ●——● PL732.**

(Rock et al, 1984)

Table 7.4 Changes observed in platelets stored in a PVC/DEHP bag, an unmodified 5 day container and one in which gas diffusion was inhibited. The pH was that observed prior to any necessary adjustment. (Two representative examples of 8 similar experiments).

Storage condition	Days in storage	Platelet ($X10^3/\mu L$)	pH	Discs (%)	Spheres (%)	Uncl (%)	pO_2 (Torr)	pCO_2 (Torr)
Sample 1	Fresh	1,210	7.02	75	25			
1.PL-146	1		7.03	80	15	<5		
	2		6.89	60	35	<5	31	51
2.CLX	1		7.26	20	40	40		
	2		7.32	10	20	70	81	43
3.CLX	1		6.51	10	85	<5		
(inhibited)	2		6.39	5	90	<5	28	70
Sample 2	Fresh	970	6.80	85	15			
1.PL-146	1		6.79	60	35	<5	22	68
	2	954	6.83	45	50	<5	39	52
2.PL-732	1		7.10	30	40	30	118	38
	2	919	7.10	10	40	50	126	28
3.PL-732	1		6.56	15	80	<5	89	68
(inhibited)	2	873	6.68	10	85	<5	91	35

(Fratantoni et al, 1984)

As a first step in determining the cause of these changes, Fratantoni et al (1984) considered the unique features of 5 day containers : a marked decrease, or total absence of DEHP, and increased gas permeability resulting in sustained higher pH and pO_2. When the pH of platelets stored in PL732 or CLX were maintained at a level below 6.5, the predominant morphology was the sphere, as is observed in the case of DEHP plasticised PVC platelet storage containers. Holme & Murphy (1983), have postulated the occurrence of two types of lesions during storage at 20-24°C in such containers. One occurs with high platelet count and is characterised by fall in pH and disc to sphere transition. The other is seen with low cell counts and is not accompanied by a fall in pH. It is characterised by an increased dispersion of cell size distribution and by loss of discoid shape, although details of the morphology have not been described. Perhaps the increased gas permeability of the 5 day containers, and the resultant higher pH and pO_2, irrespective of cell counts, simulates the low cell count type of lesion.

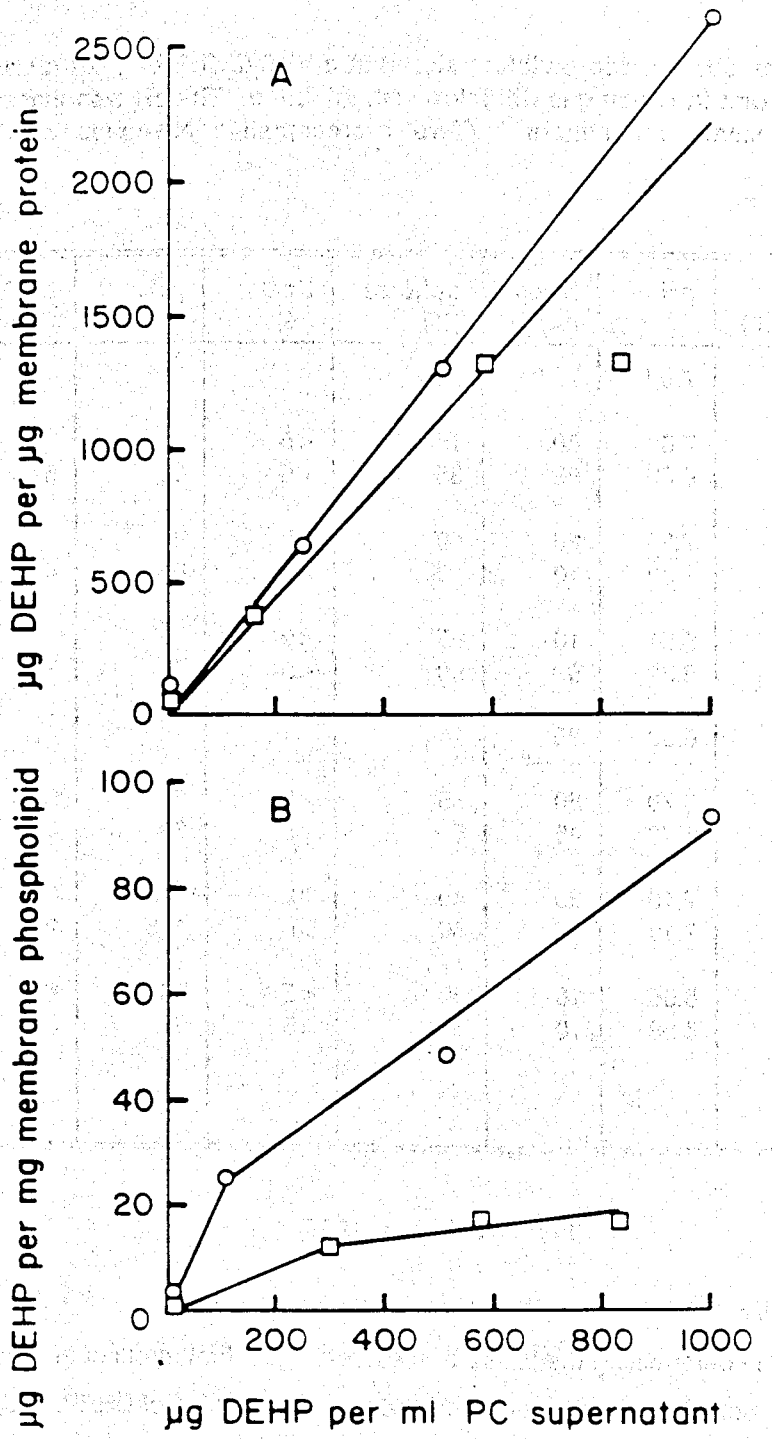


Fig 7.6 **Aliquots of PCs were removed form either the PL146 (0) or the PL732 (x 10) bag at several time intervals during storage, and the µg membrane protein (A) or µg membrane phospholipid (B) was measured and plotted as a function of µg DEHP per ml PC supernatant.**

(Labow et al, 1986)

However, while Fratantoni et al (1984) were able to abrogate the atypical changes by lowering the pH and blocking gas diffusion (table 7.4), others have reported favourable morphology and function of platelets when pH was maintained above 7.0 with potassium hydroxide, White et al (1980), or with ion exchange resins (Kotelba-Witkowska et al, 1981). Further, when Djaldette, et al (1979), incubated platelets for short periods at pH levels up to 9.0, only pseudopod formation and aggregation were seen. In the presence of a high oxygen atmosphere storage of platelet concentrates demonstrated increased incidence of atypical forms both in DEHP plasticised PVC and polyolefin containers. The appearance of increase atypical forms in platelet concentrates stored in PI-146 strongly suggests that the higher oxygen level, which results from greater permeability of the 5 day containers, is a causal factor (Fratantoni et al, 1984).

Functional deficits in platelets stored in 5 day containers on the basis of resistance to hypotonic shock and serotonin uptake assays were not observed, and satisfactory results in clinical ^{51}Cr survival and bleeding time tests have been reported (Murphy et al, 1982: Simon et al, 1983, Fratantoni et al, 1984).

The nature of the cellular changes observed suggests that cytoskeletal protein alterations may be involved. In this context Prodouz & Moroff (1983) have reported platelet cytoskeletal protein changes which are dependent on the storage conditions employed.

Labow et al (1986) assessed the effects of extractable materials on morphology and function of platelets during 5 day storage in DEHP plasticised PVC (PL146) and polyolefin (PL732) containers. They detected the presence of DEHP in the platelet concentrates stored in the polyolefin bags which are not formulated with this plasticiser, although they do contain an antioxidant 1,3,5 - trimethyl - 2,4,6-tris (3,5-di-tert-butyl-4-hydroxbenzyl) benzene (BHBB). This finding has been verified by the manufacturer (Baxter Fenwall Laboratories) who investigated the sterilisation and other processing procedures to determine the cause. Data from Labow et al (1986) indicate that the DEHP migrates from the primary pack into the polyolefin satellite during the sterilisation process in the autoclave; polyolefin bags autoclaved separately from the primary DEHP pack are free of the plasticiser.

Since DEHP has been shown to bind to the red cell membrane and protect it from haemolysis during storage, Labow et al (1986), also studied the binding of DEHP to the platelet membrane in an attempt to relate DEHP to the function and/or abnormal morphology seen in stored platelets. An earlier reference was made to the difference between the extraction by red cells and plasma of DEHP during storage, ie where the DEHP concentration was 50 percent in the cytosol and 50 percent in the membrane, the compartmentalisation in the platelets was 5:95, cytosol: membrane. The deposition of DEHP in the membrane fraction apparently depends on a functionally intact platelet. When the aggregation response was absent, DEHP freely entered the cytosol. However, when the platelets were responsive, the membrane continued to bind the plasticiser in a linear manner (fig 7.6), and this was directly proportional to the concentration of DEHP in the supernatant.

The effects of this association of DEHP with the platelet membrane are not clear. Although there is 10 to 12 times more DEHP in the plasma supernatant in platelets stored in the PVC than the polyolefin container at each time period measured, no dose response to the DEHP association with the platelet membrane could be obtained on the basis of aggregation response to paired stimuli.

The number of platelets displaying normal norphology decreased in both containers with increased storage time, however the leaf-like shapes were only seen after 48 hours in the polyolefin pack. In the study by Labow et al (1986), the platelets exposed to polyolefin for more than 48 hours showed 15 to 35 present more aberrant morphology compared to the platelets stored in PVC bags. Based on the fact that DEHP stabilises the red cell membrane, it had been considered likely that the presence of DEHP in the PVC bag might prevent the development of platelets displayed aberrant morphology but a comparative assessment was not possible due to the presence of DEHP in the polyolefin containers as reported earlier. The significance of the development of these morphological charges, while still to be fully understood, have however not been correlated with unfavourable platelet function or viability.

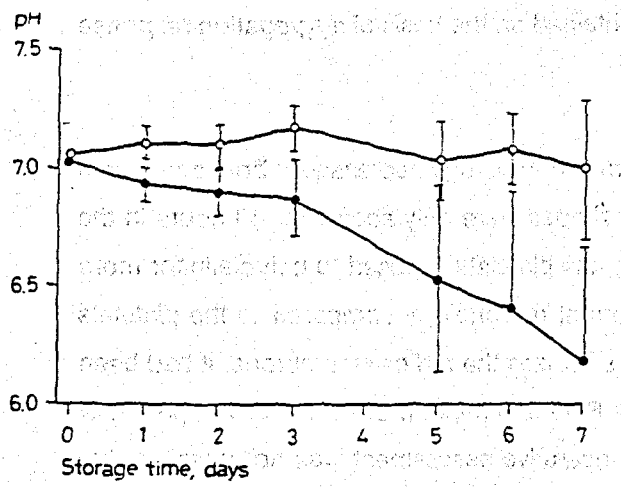


Fig 7.7. pH of platelet concentrates during storage in F76 ( ) or in F702 ( ) plastic (means ±SD; n=14).

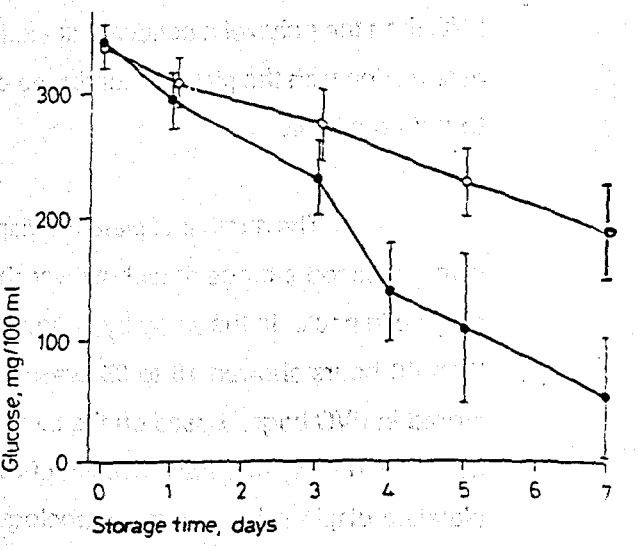


Fig 7.8. Plasma glucose concentration of platelet concentrates during storage in F76 ( ) or in F702 ( ) plastic (means ± SD; n=14)

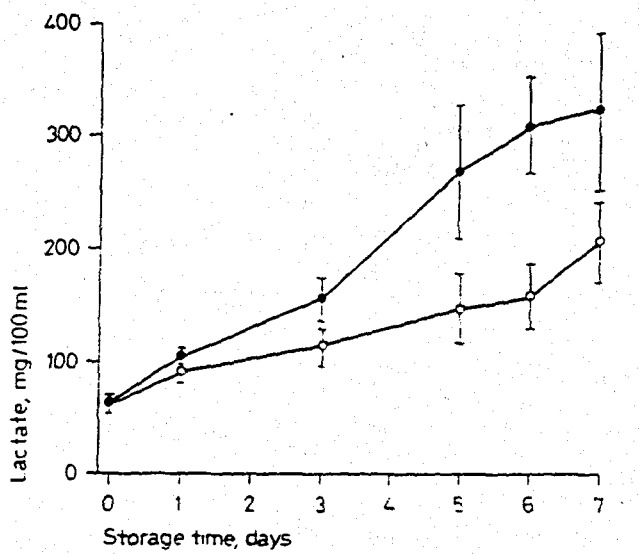


Fig 7.9. Plasma lactate concentration reaction platelet concentrates during storage in F76 ( ) or in F702 ( ) plastic (means ± SD; n=14).

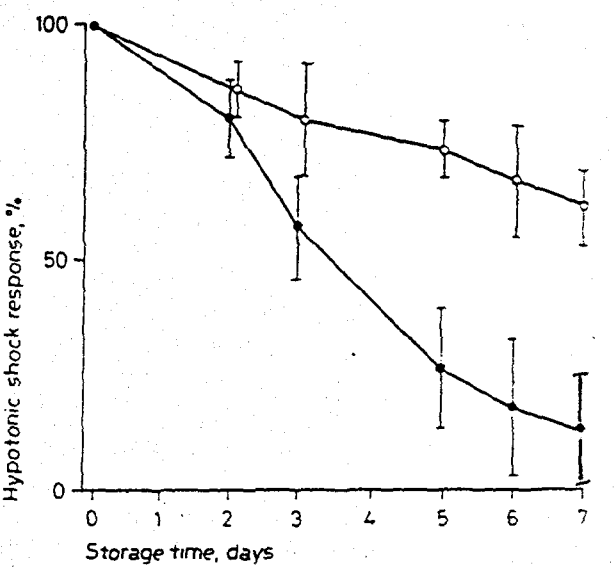


Fig 7.10. Hypotonic shock of platelet concentrates during storage in F76 ( ) or in F702 ( ) plastic (means ± SD; n=14)

(Koerner, 1984)

7.6 TET PLASTICISED PVC PLATELET CONTAINER

Koerner (1984) describes 7 day room temperature in vitro platelet studies comparing the performance of a conventional DEHP plasticised PVC container (F76) with a new PVC container (F702) which is plasticised with tri(2-ethylhexyl)-1,2,4-benzenetricarboxylate (TET). These containers were manufactured by Biotest Pharma, Frankfurt, Germany. This new material displayed increased permeability to oxygen and carbon dioxide and therefore a pH decrease does not occur during 7 days of platelet storage (fig 7.7). The decrease of plasma glucose concentration and the increase in plasma lactate in the new container is less than in the standard (fig 7.8, fig 7.9).

In vitro platelet function measured as hypotonic shock reaction (fig 7.10), aggregation response to ADP and collagen and ^{14}C-serotonine uptake (fig 7.11) was better than that displayed by the DEHP plasticised PVC material.

Platelets stored at room temperature are metabolically active and have the capacity to metabolize glycogen or glucose by glycolysis to lactate and by the Krebs cycle to carbon dioxide. It has been estimated that each of these processes accounts for half the ATP production by resting platelets during in vitro conditions (Weiss, 1975). During aerobic glycolysis, the yield of ATP from each mole glucose is 19 times that of anaerobic glycolysis (Koerner, 1984). The higher oxygen transport across the wall of the TET container favours the aerobic metabolism of stored platelets. This is seen in a decrease of the accumulation of the lactate and in a reduction of glucose consumption in the F702 plastic compound compared with F76 plastic during platelet storage. The improved platelet storage conditions in the new F702 plastic compared to standard PVC are also demonstrated in the superior platelet aggregation response, serotonine uptake and hypotonic shock reaction. A reduction in the rate of TET plasticiser extraction in comparison with DEHP (Koerner, 1984), together with recent studies of platelets stored in TET plasticised PVC platelet bags have shown that a 7 day shelf life at room temperature seems possible (Archer, et al, 1982). Similar results are also found by other investigators for platelets stored in PVC-TET plastic, measuring in vitro function or recovery and survival using ^{51}Cr labelling (Blajcman et al, 1981; Champion et al, 1981; Murphy & Holme, 1981).

Koerner (1984) concludes that the F702 platelet container will not only permit 5 day platelet storage at room temperature but will even improve the in vitro quality of platelets stored up to 72 hours when compared with standard platelet containers currently in use. The literature does not record the plasticising efficiency of TET, its cost as a raw material or indicate whether TET plasticised PVC platelet containers are able to compete with the existing systems on a cost benefit basis.

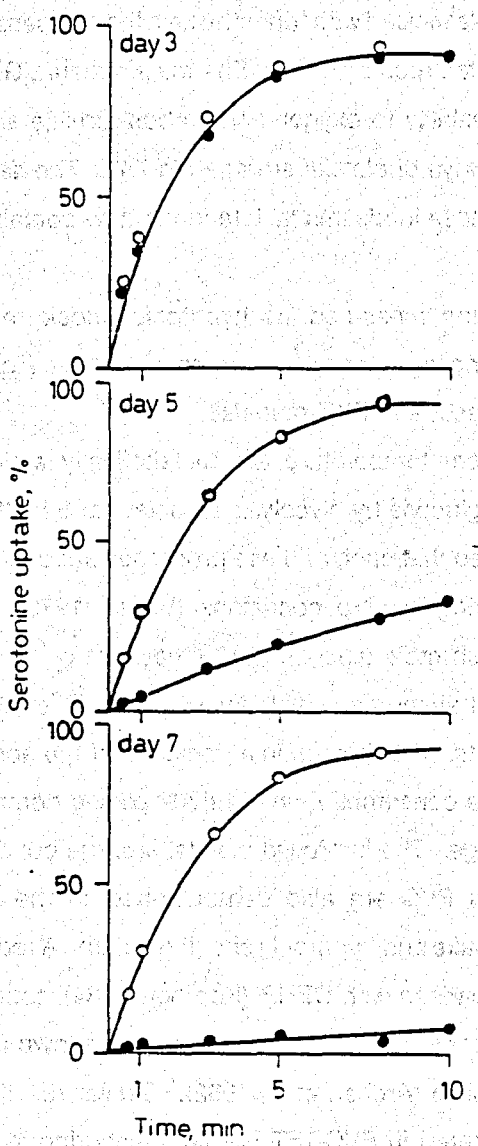


Fig 7.11. ^{14}C-Serotonine uptake of platelets after 3, 5 and 7 days of storage in F76 (●) or in F702 (○) plastic.

(Koerner, 1984)

7.7 DEHP PLASTICISED PVC PLATELET CONTAINER WITH INCREASED GAS PERMEABILITY

Holme et al (1989), have described a new container made from DEHP plasticised PVC displaying greater gas transmission properties. Compared with traditional PVC containers, it has increased gas permeability owing to a reduction in film thickness, larger surface area (400 ml capacity) and more porous label. The oxygen permeability coefficient $K(0_2)$ to the maximal platelet count, it was predicted that this maximal count would be in the range of 7.9 to 8.9×10^{10} platelets. This prediction was confirmed by carrying out 58 studies measuring pH, $p0_2$ and platelet count on platelet concentrates stored for up to 7 days (fig 7.12 and fig 7.13).

The containers for this study were supplied by Terumo Corporation (Elkton, MD) and compared against the performance of platelets stored in polyolefin bags (PL-732, Baxter Fenwal, Deerfield, IL), TOTM PVC bags (PL-1240, Baxter Fenwal), and TOTM PVC bags (CLX, Cutter Biological, Berkeley, CA).

TABLE 7.5 In Vitro results (Mean ± SD) from Platelet concentrates stored for 5 days in various containers

Containers	Number of Studies	Count (percentage of Day 1)	Total ATP (μmol/10^{11} platelets)	Extent of shape change (percentage increase OD)	HSR (percentage recovery)
PL-372	22	94 ± 4	5.6 ± 0.8	10.6 ± 3.7	70 ± 13
Terumo	21	93 ± 6	4.1 ± 2.3	14.4 ± 5.8*	39 ± 14*
CLX	4	NT**	4.6 ± 0.8	13.9 ± 2.6	59 ± 11
PL-1240	7	92 ± 5	NT	12.9 ± 2.9	64 ± 21

*** Significant difference from PL-732 container ($p<0.05$).**

**** Not tested**

(Holme et al, 1989)

Table 7.5 shows the results of in vitro evaluation of 21 platelet concentrates after 5 days of storage. There were no significant differences between platelet concentrates stored in the new container and those stored in the other containers with respect to a loss of platelet count, pH and ATP levels. However, a significant reduction in HSR was observed in platelet concentrates stored in the new container; this loss did not occur at the start of the storage but developed with increasing storage time. The ADP-induced platelet shape change response, reflecting the extent of disc to sphere transformation was maintained significantly better in the new container than in the PL-732 container.

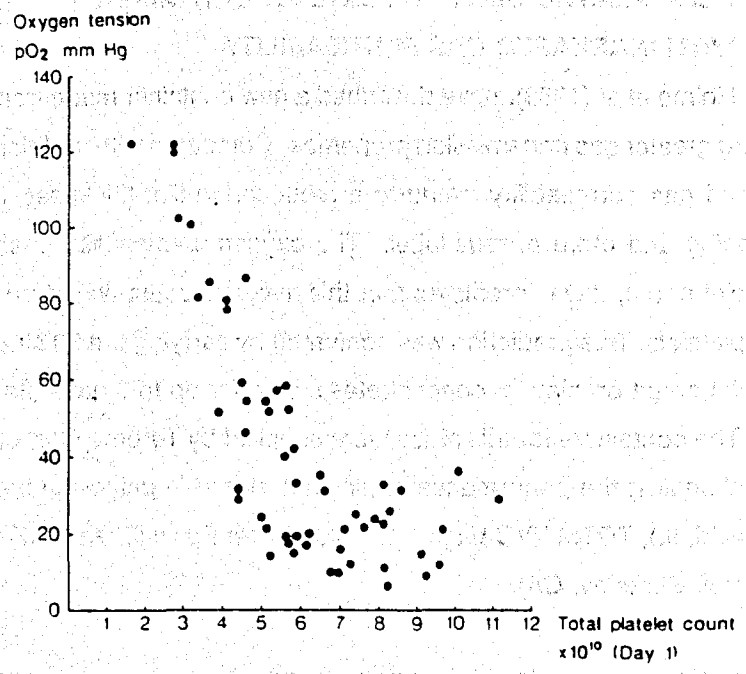


Fig 7.12. Total platelet count in the containers compared to oxygen tension, pO_2 in torr of the platelet concentrate measured at Day 1 of storage on 58 PCs. An inverse linear relationship (r = -0.86) between total count and pO_2 levels is apparent at counts below 8.0 x 10_{10} platelets.

(Holme et al, 1989)

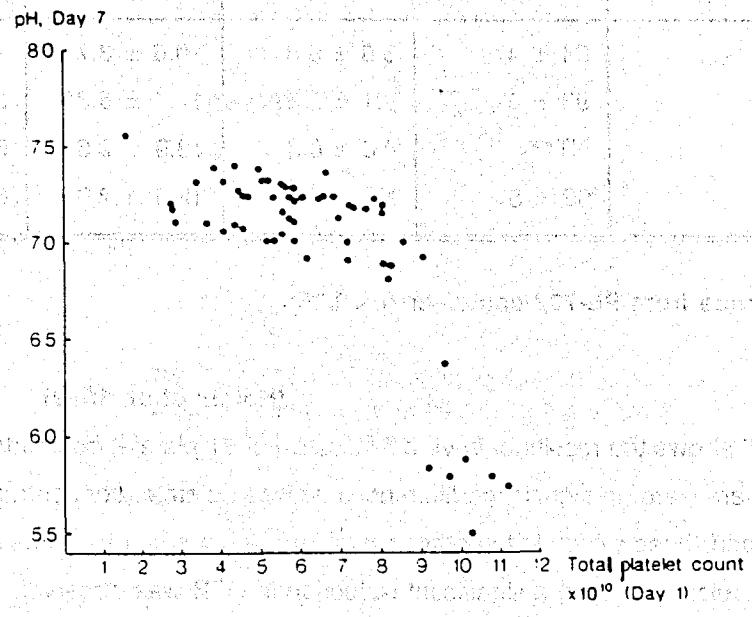


Fig 7.13. Total platelet count in the container compared to the pH of the platelet concentrate measured at Day 7 on the same 58 PCs shown in figure 1. A marked fall in pH is apparent in PCs with counts higher than 8.0 x 10^{10} platelets.

(Holme et al, 1989)

The maintenance of discoid structure and the HSR are in vitro determinants that have been shown to correlate with in vivo viability (Turner et al, 1974; Valeri et al, 1974; Holme et al, 1978; Estep et al, 1984). In previous studies Holme et al (1987) also found a high correlation between these two determinants, but this was not the case in the present study where the platelet discoid structure was well maintained, but the HSR was markedly reduced during storage. This study showed that a reduction in HSR is not necessarily related to a reduction in post transfusion survival, when using Indium-III-oxine as the radiolabel post transfusion survival studies performed on 8 units stored for 5 days recorded percentage recoveries averaged at 47.5 $\pm$ 6.2 percent and a mean survival of 155 $\pm$ 33 hours. The marked reduction in HSR was thought due to the continuous extraction of DEHP plasticiser by the platelet concentrates during storage. This was assessed by high performance liquid chromotography using the method described by Shintani et al (1984) and shown in fig 7.14.

The studies by Home et al (1989) and showed that the oxygen permeability of DEHP plasticised thinner walled platelet containers allowed for acceptable pH levels in 90 percent of the stored platelet concentrates having counts below 9 x 10" for 5 days of storage. While the DEHP extraction was associated with a reduction in HSR, normal platelet morphology was preserved, and the in vivo recoveries and survivals deemed satisfactory.

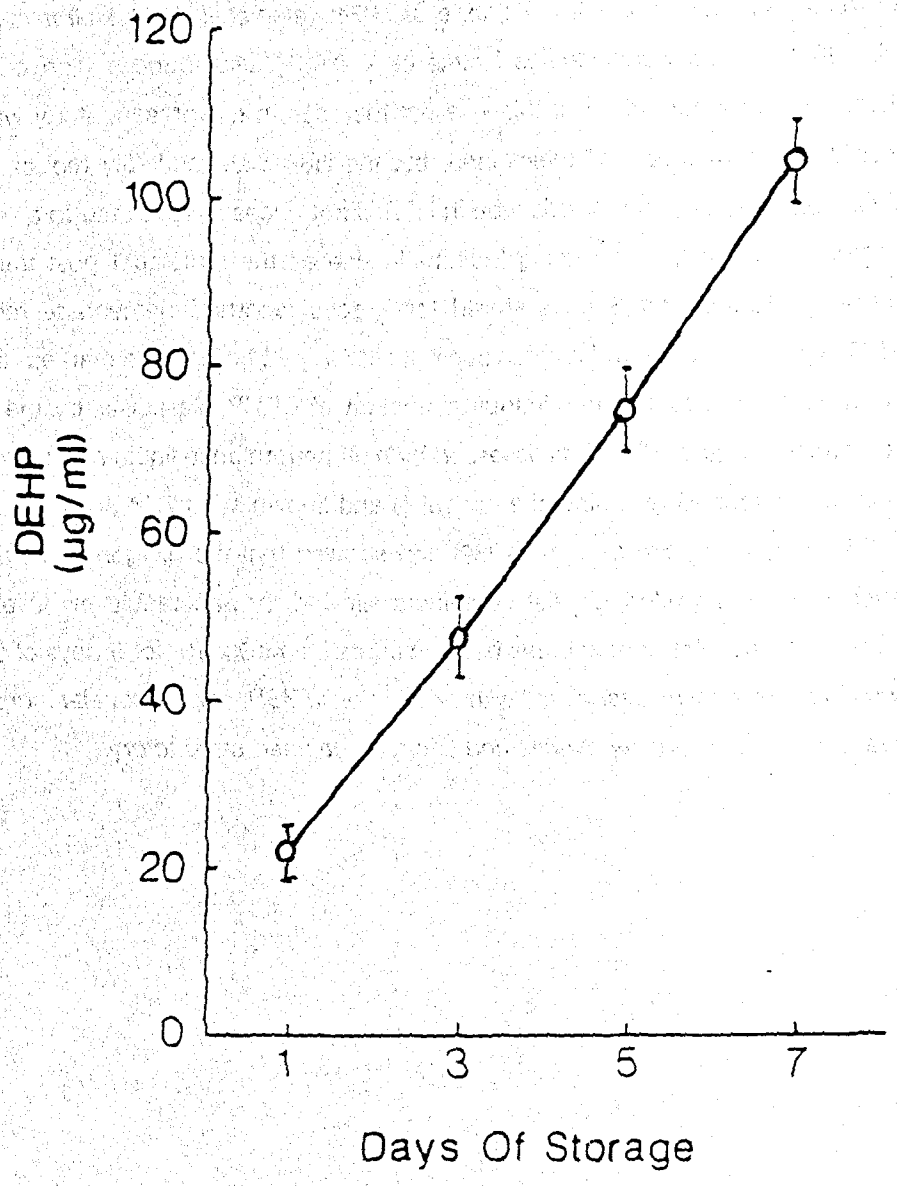


Fig 7.14. Release of DEHP from the container into the PCs during 7 days of storage. Means ± SD of eight studies are plotted.

(Holme et al, 1989)

CHAPTER 8

CHAPTER 8

FUTURE PROSPECTS FOR BLOOD STORAGE IN POLYMERIC BIOMATERIALS.

8.1 THE DEHP CONTROVERSY.

In considering the importance of flexible PVC materials applied to blood collection, storage and delivery, attention has focused on the properties and performance of DEHP, the major plasticiser employed. During the last decade, a number of important in-vitro studies have demonstrated that the storage of red blood cells in the presence of DEHP results in better retention of normal cell morphology, enhanced osmotic stability and lower haemolysis than is observed during storage in the absence of this plasticiser (Estep et al 1984; Rock et al, 1984; Horowitz et al, 1985). While most of this plasticiser extracted from flexible PVC blood bags accumulates in plasma, approximately 5%-10% is associated with red blood cells when whole blood is stored under typical blood bank conditions. These authors demonstrated that the extracted DEHP binds to the red cell membrane and stabilises it by lowering its osmotic fragility. After 14 days, the osmotic fragility and plasma-free haemoglobin increase much more rapidly when red cells are stored in non-DEHP plasticised containers. Osmotic fragility and plasma-free haemoglobin have been correlated directly with DEHP concentration during storage. The stability of red blood cells in non-DEHP plasticised containers could not be maintained beyond 21 days at 4-6°C. Decreased flexibility of red blood cells has been shown to result directly from the loss of lipid from the membrane surface. It has therefore been suggested that the protection by DEHP may be due to in incorporation of the very hydrophobic DEHP into the area of the red blood cell membrane that has been vacated by the lipid.

The effect of DEHP on in-vivo red cell survival has been the subject of a more recent study (AuBuchon et al, 1988). Experiments were conducted to address the issue and ascertain whether the previous in vitro observations were indicative of a clinically relevant improvement in red cell function. The post-transfusion survival of red blood cells stored under refrigeration for 35 days in CPDA - 1 anticoagulant in DEHP plasticised PVC containers was 17-24% higher than the survival of cells stored in containers plasticised with TOTM. Enhanced survival was also observed when DEHP was added to whole blood stored in glass bottles offering further evidence that the improved post-transfusion survival was indeed attributable to the presence of this plasticiser.

The conclusion drawn following both in vitro and in vivo studies suggests that the removal of DEHP from such systems could result in decreased red cell post-transfusion survival if no compensatory additives were used to replace the protective effects of DEHP.

In the case of platelet survival, DEHP plasticised PVC containers which have been the subject of extensive studies (Rock et al 1984) do not demonstrate an advantage over other systems in current use, although due to its inherent cost advantage 3 day platelet containers currently meet ca 90% of total demand on a global basis (Baxter Fenwal, personal communication).

The evaluation of human health risks and effects of DEHP on the environment has been considered in a recent publication under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organisation (WHO, 1992). They reported that almost all the DEHP present in the environment arises from anthropogenic sources rather than from natural ones. The worldwide production of DEHP has been increasing during recent decades and at present amounts to about 1×10^6 tonnes per year. One third of total production is in the USA and one third in Europe. Discarded plasticised products may be disposed of either by incineration, the recommended method for post hospital waste products, or via dumping in landfill sites. During incineration at a low temperature, a large percentage of the DEHP may be lost to the atmosphere. The environmental fate of DEHP in landfill sites has not been well studied and no definite conclusions can be drawn, although the exposure to DEHP from drinking water and food is low. DEHP concentrations of up to $300ng/m^3$ have been measured in urban air, but usually the levels in ambient air are well below $100ng/m^3$. DEHP is readily photodegraded in the atmosphere, but in the environment aerobic biodegradation seems to be slow and anaerobic degradation appears to be even slower. Clinical treatment, including transfusion, haemodialysis, extracorporeal circulation, and artificial respiration, may lead to increased DEHP exposure.

The WHO report recommendations for protection of human health and the environment state that current disposal practices should be improved, measures must be taken to reduce the release of DEHP to the environment, and that medical devices and products that contribute to the body burden of DEHP must be scrutinised to reduce exposure to DEHP via the intravenous route. They conclude that more research is needed on the effects of DEHP on the ecosystem of sediments and on the subject of DEHP leaching, particularly from landfill to ground water, further inhalation studies should be performed and epidemiological surveys on exposed populations should be encouraged. Finally, further studies are needed on the mechanism of peroxisome-proliferator-induced heptocarcinogenicity, with emphasis on human health effects.

8.2 CITRIC ACID ESTERS

The search for a suitable replacement for DEHP, which demonstrates a lower order of medium term toxicity while imparting to PVC the same range of desirable properties has focused attention on a group of citric acid esters. Citric acid, which occurs naturally in citrus fruits, appears to be an ideal precursor for this purpose. It is readily available commercially and affords four sites for esterification. This feature in turn provides a high degree of freedom in designing the optimum molecular mass and polarity relationship needed to achieve the correct balance of properties in a plasticised PVC formulation.

Table 8.1 lists a number of citric acid esters which have been employed as plasticisers in films for food contact applications over the past four decades.

Table 8.1 Citric acid ester plasticisers

Generic Name	Molecular Mass	Application
Triethyl Citrate	276	Cellulosics
Acetyltriethyl Citrate	318	Cellulosics
Tri-n-butyl Citrate	360	Cellulosics
Acetyltri-n-butyl Citrate	402	PVC, PVDC

The acetyltributyl citrate (ATBC) has been accepted for use as a polymer in plastic films for packaging foodstuffs by the FDA (Lehman, 1956). This citrate is the preferred plasticiser for food packaging due to its pharmacological safety, economy, performance and lack of odour. A summary by Wilson & McCormick (1954) of toxicity studies of plasticisers accepted by the FDA has shown that ATBC was one of the safest compounds reported at that time. It is an efficient plasticiser and demonstrates excellent compatibility with PVC over a wide Shore A hardness range. ATBC is however readily extracted by lipids, which limits its use in flexible PVC compounds for medical device applications. From a large number of citric acid esters with a higher molecular mass prepared and screened for compatibility two candidates emerged namely acetyltri-n-hexyl citrate (ATHC) and n-butyryltri-n-hexyl citrate (BTHC) with molecular mass of 486 and 514 respectively.

The storage stability of whole blood and of platelets stored in bags made from BTHC plasticised PVC was studied by Kevy et al (1985) and compared with standard DEHP plasticised blood bags. They demonstrated that the BTHC plasticised PVC containers maintain the in vitro integrity of the red cells over a 49 day storage period in a similar way to the conventional container and that platelets were also well preserved.

Recent studies on the acute toxicity of BTHC were conducted by the Drug Safety Evaluation Department of Pfizer, Inc. and reported by Guiot et al, (1992). The oral administration of maximum practical dose volumes (>48g/kg in mice and >20g/kg in rats) of BTHC produced no signs of systemic toxicity and no mortality in fasted mice or rats. On

rabbits, tests indicate that undiluted BTHC is not a primary skin irritant nor ocular irritant to unrinsed eyes. At the levels tested, BTHC does not induce gene mutation in either microbial cells or mammalian cells in vivo or chromosomal mutation in vitro.

Previous reports by Moody & Reddy (1978) and by Warren et al (1982), demonstrated that rats fed with a DEHP containing diet resulted in hepatomegaly, increased hepatic activity of catalase and carnitine acetyltransferase and a decrease in serum lipid in association with hepatic peroxisome proliferation. These findings were confirmed by Kevy et al (1987). Those rats fed either a normal diet or one containing 3% w/w BTHC did not demonstrate peroxisome proliferation and maintained normal biochemical parameters. In summary DEHP fed animals demonstrated alterations of hepatic architecture by light microscopy, marked peroxisome proliferation, increased peroxisome enzymes and hypolipidemia. BTHC when administered for 5 weeks to a rat, does not induce hepatic peroxisome proliferation or otherwise alter the structure of hepatocytes. The results were identical to rats fed a normal diet. The citrate BTHC was hydrolysed on incubation with rat serum, liver or interstinal enzyme preparations. However of a possible three moles of hexanol for every citrate mole, only one hexanol mole was initially liberated. Hydrolysis proceeds faster in serum than in liver and is much slower in the interstinal preparation.

A new PVC blood bag was recently introduced by the Baxter Healthcare Corporation under the trade mark PL2209 and formulated on the BTHC plasticiser previously described. As this plasticiser is extracted to a much lesser extent by contact with biological fluids being less soluble than DEHP (Hull et al, 1984), and the products of its hydrolysis by esterases found in blood and other tissues are citrate, butyrate and hexanol which are naturally occurring and readily metabolised, unlike MEHP the principal and toxic metabolite of DEHP, it demonstrates major benefits.

A useful model for testing the toxicity of compounds using human tissue is the human atrial trabecular preparation which has been applied by Barry et al (1990), to assess the effects of DEHP and MEHP. Applying this technique to BTHC, Labow et al (1992), concluded that it demonstrates a reduced interaction with the biological system and thereby diminishes a potential toxic response.

It is too early to predict to what extent the use of BTHC will replace DEHP as a plasticiser for flexible PVC blood bag containers. Providing it can be offered without incurring excessive cost its undoubted benefits should ensure a promising future. If the present high cost of BTHC which is comparable with TOTM and PA plasticisers prevails, its use will be restricted to specialist requirements or in those markets where regulation currently restricts the use of DEHP plasticised PVC for blood contact applications.

8.3 IN CONCLUSION

The review demonstrates the important role performed by plasticised poly(vinyl chloride) compositions in relation to the collection, storage and delivery of blood and blood components. It owes its success to ease of fabrication and device assembly, mechanical performance in use, inherent safety and known cost benefits. Studies on alternative polymers for plasticised poly(vinyl chloride) will need to be similarly well researched and demonstrate improved performance before gaining wide acceptance.

BIBLIOGRAPHY

Adams GA, Swenson SD, Rock G (1986)
Survival & recovery of human platelets stored for five days in a non plasma medium.
Blood 67 : 672-675

Albro PW, Corbett JT, Schroeder JL, Jordan S, Mathews HB (1982)
Pharmacokinetics, interactions with macromolecules & species differences in metabolism of DEHP.
Environ. Health Perspect. 45 : 19

Albro PW, Thomas R, Fishbein L (1983)
Metabolites of dietary di ethyl-hexyl phthalate in rats. Isolation & characterisation of the urinary metabolites.
J. Chromatogr. 76 : 321

Archer GT, Grimsley PG, Jindra J, Robson JE, Ribeiro A (1982)
Survival of transfused platelets collected into new formulation plastic packs.
Vox Sang. 43 : 223-230

Au Buchon JP, Davey RJ, Estep T, Miripol J (1984)
Effect of the plasticizer di 2-ethyl hexyl phthalate on survival of stored red cells.
Transfusion 37 : 422, (abstr)

AuBuchon JP, Estep TN, Davey RJ (1988)
The effect of the plasticizer di (2-ethyl hexyl) phthalate on the survival of stored RBCs.
Blood 71 : 448- 452

Barry YA, Labow RS, Tocchi M, Keon WJ (1990)
Toxicol Appl Pharm 106, 48-52

Becker GA, Tuccelli M, Kunichi T, Chalos MK, Aster RH (1973)
Transfusion 13 : 61-68
Biggs MS, Blass CR (1987)
Medical applications for extraction resistant PVC compounds - Medical Plastics '87 Conference, Copenhagen, Denmark, 15 September 1987.

Blagdon J (1972)
The long-term storage of blood by freezing.
British Journal of Hospital Medicine & Equipment
Supplement 8 : 24-34

Blajcman MA, Kelton JG, Senyi AF, Klein C, George J (1981)
The maintenance of haemostatic function, survival, platelet membrane glycoproteins & pH of human platelet concentrates stored for 5 days in a new polyvinyl chloride blood bag.
Blood 58 : suppl. 1, p 178a

Blass CR (1990)
Medical Applications for extraction resistant PVC compounds
In: Progress in Biomedical Polymers
Ed: Gebelein CG & Dunn RL, Plenum Press, New York 1990

Blass CR, Biggs MS, Jones C, Courtney JM, Lowe GDO (1991)
Blood response to plasticised poly (vinyl chloride).
Plastics, Rubber and Composites Processing and Appn. 15 : 221-227

Blass CR (1992)
In: Medical Device Technology. Ed: Young CE, Aster Publishing Corp., USA, Vol 3, 3 : 32-40

Bowry SK, Courtney JM, Prentice CRM, Douglas JT (1984)
Utilization of the platelet release reaction in the blood compatibility assessment of polymers.
Biomaterials 5 : 289-292

Bowry SK, Prentice CRM, Courtney JM (1985)
A modification of the Wu and Hoak method for the determination of platelet aggregates and platelet adhesion.
Thromb. Haemostas, 53 : 381-385

Card RT, Mohandas N, Perkins HA, Shohet SB (1982)
Deformability of stored red blood cells. Relationship to degree of packing.
Transfusion 22 : 96

Card RT, Mohandas N, Mollison PL (1983)
Relationship of post transfusion viability to deformability of stored red cells.
Br. J. Haematol. 53 : 237

CEFIC (1985)
DEHP - A critical review & interpretation of the available toxicological information. CEFIC, Brussels.

Champion AB, Wada S, Ladehoff D, Carmen RA (1981)
Effect of blood bag material on in vitro platelet function during six days of storage at 22°C.
Blood 58 : suppl.1, p 179a

Chenoweth DE (1984)
Complement activation during hemodialysis. Clinical observations, proposed mechanism, and theoretical implications.
Artif. Organs, 8 : 281-287

Christnesson A, Ljunggren L, Nilsson-Thorell C, Arge B, Diehl U, Hagstam KE, Lundberg M (1991)
In vivo comparative evaluation of haemodialysis tubing plasticised with DEHP & TEHTM.
Int. J. Artif. Organs 14 : 407-410

Chu I, Villeneuve DC, Secours V, Franklin C, Rock G, Viau A (1978)
Metabolism and tissue distribution of mono(2-ethyl hexyl) phthalate in the rat.
Drug Metab. Dispos 6 : 146-149

Chuang HYK, King WF, Mason RG (1978)
Interaction of plasma proteins with artificial surfaces: protein absorption isotherms
J. Lab. Clin. Med., 92, 483-498

Clark MR, Mohandas N, Shohet SB (1983)
Osmotic gradient ektacytometry : Comprehensive characterization of red cell volume & surface maintenance.
Blood 61 : 899

Cole RS, Tocchi M, Franklin CA, Villeneuve DC, Rock G (1979)
The enzymatic conversion of DEHP to MEHP in human plasma
XIth Int. Conr/ Biochem; Toronto 1979.

Cole R, Tocchi M, Posen G, Smiley RK, Villeneuve DC, Rock GA (1980)
Blood levels of plasticisers in hemophiliacs and hemodialysis patients : the multi-transfused patient (abstr.). Presented to International Society of Hematology International Society of Blood Transfusion, Montreal, August 1980.

Cole RS, Tocchi M, Wye E, Villeneuve DC, Rock G (1981)
Contamination of commerical blood products by di-(2-ethyl hexyl) phthalate.
Vox Sang 40 : 317-322

Contreras TJ Sheilby RH, Valeri CR (1974)
Accumulation of di(2-ethlyhexyl)phthalate (DEHP) in whole blood, platelet concentrates and platelet poor plasma.
Transfusion 14 : 34-46

Courtney JM, Travers M, Bowry SK, Prentice CRM, Lowe GDO, Forbes CD (1987)
Measurement of platelet loss in the blood compatibility assessment of biomaterials.
Biomaterials 8 : 231-233

Courtney JM, Irvine L, Jones C, Mosa S, Robertson LM, Srivastava S (1989 a)
Biomaterials - a bioengineering prospective
Int. J. Artif. Organs, in press

Courtney JM, Robertson LM, Jones C, Irvine L, Douglas JT, Travers M, Ryan CJ, Lowe GDO (1989 b).
Blood compatibility of biomaterials in artificial organs.
in Progress in Bioengineering, (eds) JP Paul, JC Barbenel, JM Courtney, RM Kenedi, Adam Hilger Press Bristol pp 21-27

Danon D, Frei YF, Rimon A, Ben-David A (1964)
Simple rapid osmotic fragility test proposed as a routine in blood banks.
Transfusion 4 : 339-342

Dern RJ, Gwinn RP, Wiorkowski JJ (1966)
Studies on the preservation of human blood. 1. Variability in erythrocyte storage characteristics among healthy donors.
J. Lab. Clin. Med. 67 : 955-965

Djaldette M, Fishman P, Bessler H, Chaimoff C (1979)
pH-Induced platelet ultrastructural lesions.
Arch. Surg. 114 : 707-710

Elcombe CR, Mitchell AM (1986)
Peroxisome proliferaiton due to di (2-ethyl hexyl) phthalate (DEHP). Species differences & possible mechanisms.
Environ. Health Perspect. 70 : 211

Estep TN, Pederson RA, Miller TJ, Stupar KR (1984)
Characterization of erythrocyte quality during the refrigerated storage of whole blood containing di (2-ethyl hexyl) phthalate.
Blood 64 : 1270-1276

Flaminio LM, Bergia R, De Angelis L, Ferazza M, Marinovich M, Galli G, Galli CL (1988)
Leachability of a new plasticiser tri-(2-ethyl hexyl) trimellitate from haemodialysis tubing.
Int. J. Artif. Organs 11 : 435-441

Forbes CD, Courtney JM (1987)
Thrombosis & artificial surfaces.
in Haemostasis & Thrombosis, ed A.L. Bloom & D.P. Thomas, Churchill Livingstone, Edinburgh, UK, 1987, pp 902-921

Fratatoni JC, Studivant B, Poindexter BJ (1984 a)
Aberrant morphology of platelets stored in 5 day containers.
Thromb. Res. 33 : 607-615

Fratantoni JC, Poindexter BJ, Bonner RF (1984 b)
Quantitative assessment of platelet morphology by light scattering. A potential method for the evaluation of platelets for transfusion.
J. Lab. Clin. Med. 103 : 620-631

Garvey LK, Swenberg JA, Hamm TE, Popp JA (1987)
Di(2-ethyl hexyl) phthalate: lack of initiating activity in the liver of female F-344 rats.
Carinogenesis 8 : 285

Gibson TP, Briggs WA, Boone BT (1976)
Delivery of di (2-ethyl hexyl) phthalate to patients during haemodialysis.
J. Lab. Clin. Med. 87 : 519-524

Gilbo CM, Coles NW (1975)
Di(2-ethyl hexyl) phthalate content of plasma fractions prepared by low temperature ethanol (Cohn) fractionation.
Vox Sang. 29 : 242-247

Greenwalt TJ, Perry S (1969)
Preservation & utilization of the components of human blood. In Progress in Haematatology, ed. Brown, E. B & More, C.V. Vol VI, pp 148-180. London : Heinemann Medical.

Guiot P, Ryan MA, Hull EH (1992)
Food, pharmaceutical & biomedical applications of citric acid esters
In: Chimicaoggi. 1, 53-56

Gunson HH, Merry AH, Makar Y, Thomson EE, Carter AC (1983)
Five day storage of platelet concentrates. II. In vivo studies.
Clin. Lab. Haemat. 5 : 287-294

Haradin AR, Weed RI, Reed CF (1969)
Changes in physical properties of stored erythrocytes. Relationship to survival in vivo.
Transfusion 9 : 229

Hardeman MR (1975)
The effect of continuous dialysis on platelet preservation.
Transfusion 15 : 231-236

Hayhurst EG, Wyman M (1975)
Morbidity associated with prolonged used of polyvinyl feeding tubes
Amer. J. Dis. Children 129 : 72-74

Heaton WAL (1986)
Enhancement of cellular elements. In: Wallas CH, McCarthy LJ, eds. New frontiers in blood banking.
Arlington : American Association of Blood Banks, 1986 : 89-125

Hogman CF, Arro E, Hedlun K (1980)
Red blood cell preservation in protein poor media. 2. Studies of changes in cell shape during storage.
Haematologia 13 : 135

Hogman CF, de Verdier CH, Ericson A, Hedlund K, Sandhagen B (1985)
Studies on the mechanism of human red cell loss of viability during storage at +4°C in vitro. 1. Cell shape & total adenylate concentration as determinate factors for post transfusion survival.
Vox Sang 48 : 257

Holme S, Vaidja, Kalpana, Murphy S (1978)
Platelet storage at 22°C : effect of type of agitation on morphology, viability & function in vitro.
Blood 52 : 425

Holme S, Murphy S (1983)
Platelet storage at 22°C for transfusion : interrelationship of platelet density & size, medium pH, & viability after in vivo infusion.
J. Lab. Clin. Med. 101 : 161-174

Holme S, Heaton WA, Courtright M (1987)
Platelet storage lesion in second-generation containers : correlation with platelet ATP levels.
Vox Sang. 53 : 214-220

Holme S, Heaton A, Momoda G (1989)
Evaluation of a new, more oxygen-permeable polyvinyl chloride container.
Transfusion 29 : 159-165

Horowitz B, Stryker MH, Waldman AA, Woods KR, Gass JD, Drago J (1985)
Stabilization of red cells by the plasticizer di ethyl hexyl phthalate.
Vox Sang 48 : 150-155

Hull EH, Mathur KK (1984)
High molecular weight citric acid esters as effective plasticisers for medical-grade PVC
In: Modern Plastics; McGraw-Hill, Inc, 5

Hustin A (1914)
Prinipe d'une nouvelle méthode de transfusion.
J. Méd. Brux 2 : 436

Ishikawa Y, Honda K, Sasakawa S, Hatada K, Kobayashi H (1983)
Prevention of leakage of di (2-ethyl hexyl) phthalate from blood bags by glow discharge treatment and its effects on aggregatability of platelets.
Vox Sang 45 : 68-76

Jaeger RJ, Rubin RJ (1970)
Contamination of blood stored in plastic packs.
Lancet 2 : 151

Jaeger RJ, Rubin RJ (1972)
Migration of a phthalate ester plasticiser from polyvinyl chloride blood bags into stored human blood and its localisation in human tissues.
N. Eng. J. Med. 287 : 1114-1118

Jaeger RJ, Rubin RJ (1973)
Di (2-ethyl hexyl) phthalate : A plasticizer contaminant of platelet concentrates.
Transfusion 13 : 107-108

Jones C (1989)
Blood response to plasticised poly (vinyl chloride), PhD thesis, University of Strathclyde, Glasgow.

Kevy SV, Jacobson MS, Kim B, Chao FC (1985)
A new citrate plasticiser for polyvinyl chloride (PVC); Paper presented at the American Society of Hematology, Dec 7-10, 1985

Kevy SV, Jacobson MS, Ausprunk D, Kaplan M (1987)
Peroxisome testing of Citroflex B6
Unpublished data

Klinkmann H, Wolf H, Schmitt E (1984)
Definition of biocompatibility.
Contr. Nephrol. 37 : 70-77

Klinkmann H, Falkenhagen D, Courtney JM (1987)
Clinical relevance of biocompatibility - the material cannot be divorced from the device. In: Uremia Therapy. Ed: Garland HJ, Springer-Verlag 125-140

Kluwe WM, McConnell EE, Huff JE, Haseman JK, Douglas JF Hartwell WV (1982)
Carcinogenicity testing of phthalate esters and related compounds by the National Toxicological Program and National Cancer Institute.
Environ. Health Perspect 45 : 129-133

Koerner K (1984)
Platelet function of room temperature platelet concentrates stored in a new plastic material with high gas permeability.
Vox Sang 47 : 406-411

Kotelba-Witkowska B, Harmening-Pittiglio DM, Schiffer CA (1981)
Storage of platelet concentrates using ion exchange resin charged with di basic phosphate.
Blood 58 : 537-543

Kunicki TJ, Tuccelli M, Becker GA, Aster RH (1975)
A study of variables affecting the quality of platelets stored at room temperature.
Transfusion 15 : 414

Labow RS, Tocchi M, Rock G (1986)
Platelet storage. Effects of leachable materials on morphology & function.
Transfusion 26 : 351-357

Labow RS, Card RT, Rock G (1987)
The effect of the plasticiser di (2-ethyl hexyl) phthalate on red cell deformability.
Blood 70 : 319 - 323

Labow RS, Ayres D, Watson M, Keon WJ (1992)
Assessment of the toxicity of a new plasticiser, Butyryl-trihexyl-citrate, at "Fourth World Biomaterials Congress", Berlin, April 24-28, 1992

Lake BC, Gangolli SD, Grasso P, Lloyd AG (1975)
Studies on the hepatic effects of orally administered di 2-ethyl hexyl phthalate in the rat.
Toxiol. Appl. Pharmacol 32 : 355-367

Lawrence WH (1978)
Phthalate esters : the quesiton of safety.
Clin. Toxicol 13 (1) : 89-139

Lehman AJ (1956)
In: Assoc. Food & Drug Officials U.S., Quart.
Bull 20, 159

Lewandowski M, Fernandes J, Chen TS (1980)
Assessment of the teratogenic potential of plasma soluble extracts of diethyl hexyl phthalate plasticised polyvinyl chloride plastics in rats.
Toxicol. Appl. Pharmacol 54 : 141-147

Lewis LM, Flechtner TW, Kerkay J, Pearson KH, Nakamoto S (1978)
Bis (2-ethyl hexyl)phthylate concentrations in the serum of hemodialysis patients.
Clin. Chem. 24 : 741-746

Maltoni C, Lefemine G, Ciliberti A, Carretti D (1984)
Experimental research on VCM carcinogenesis, in Archives of Research on Industrial Carcinogenesis, Vol II, ed Princetown Scientific Publishers, Princetown.

Marcel YL, Noel SP (1970)
Contamination of blood stored in plastic packs.
Lancet 1 : 35 - 36

Marcel YL (1973)
Determination of di(2-ethylhexyl)phthlalte levels in human blood plasma and cryoprecipitates.
Environ. Health Perspect. 3 : 119 - 121

Mitchel AM, Lhuguenot JC, Bridges JW, Elcolme CR (1985)
Identification of the proximate peroxisome proliferator(s) derived from di (2-ethyl hexyl) phthalate.
Toxicol. Appl. Pharmacol. 80 : 23

Mohandas N, Phillips WM, Bessis M (1979)
Red blood cell deformation & haemolytic amemias.
Semin. Hematol. 16 : 95

Mollison P L (1972)
"Blood Transfusion in Clinical Medicine" 5th ed.
Oxford : Blackwell Scientific Publications.

Moody DE, Reddy JK (1978)
Hepatic peroxisome (microbody) proliferation in rats fed plasticisers and related compounds.
Toxicol Appl. Pharmacol 45 : 497-504

Murphy S, Sayar SN, Gardner FH (1970)
Storage of platelet concentrates at 22°C.
Blood 35 : 549-557

Murphy S, Gardner FH (1971)
Platelet storage at 22°C; metabolism, morphologic & functional studies.
J. clin. Invest 50 : 370-377

Murphy S, Sayar SN, Abdou NL, Gardner FH (1974)
Platelet preservation by freezing. Use of dimethylsulphoxide as cryoprotective agent.

Murphy S, Gardner FH (1975)
Platelet storage at 22°C : role of gas transport across plastic containers in maintenance of viability.
Blood 46 : 209-218
Transfusion 14 : 139

Murphy S, Gardner FH (1976)
Room temperature storage of platelets.
Transfusion 16 : 2

Murphy S, Holme S (1981)
Storage of platelets for five days in two containers - value of paired studies.
Transfusion : 21 637

Murphy S, Kahn RA, Holme S, Phillips GL, Sherwood W, Davisson W, Buchholz DH (1982)
Improved storage of platelets for transfusion in a new container.
Blood 60 : 194-200

Murphy S, Holme S, Nelson E, Carmen R (1984)
Paired comparison of the in vivo & in vitro results of storage of platelet concentrates in two containers.
Transfusion 24 : 31-34

Murphy S (1985a)
Platelet storage for transfusion.
Semin. Hematol. 22 : 165-177

Murphy S (1985b)
Evolution, current State of Art & further trends of platelet preservation.
Abstracts, 19th Congr. Int. Soc. Blood
Transut; Sydney, p. 78.

Nikonorov M, Mazur H, Piekacz H (1973)
Effect of orally administered plasticisers and polyvinyl chloride stabilisers in the rat.
Toxicol. Appl. Pharmacol 25 : 253

Odink J (1974)
Quantitation of the response of platelet suspension to hypotoxic stress. In: Valeri CR, Hogman CF, Krijnen HW, eds. Platelet preservation & transfusion international symposium. Istanbul, Turkey: International Society of Blood Transfusion, 1974 : 59.

Onda H, Kodama H, Yamanda N, Ota H (1976)
Effect of phthalate esters on reproductive performance in the rat.
Nippon Eiseigaku Zasshi 31 : 507

Peck CC, Odom DG, Friedman HI, Albro PW, Hass JR, Brady JT, Jess DA (1979)
Di(2-ethylhexyl/phthalate (DEHP) and mono (2-ethylhexyl)phthalate (MEHP) accumulation in whole blood and red cell concentrates.
Transfusion 19 : 137-146

Produouz KN, Moroff G (1983)
Alterations in platelet cytoskeletal proteins during storage of platelet concentrates.
Transfusion 23 : 427 (abstract)

Reel JR, Wolkowski-Tyl R, Lawton AD, Lamb JC (1984)
'Diethyl hexyl phthalate (DEHP); Reproductive and fertility assessment in CD-1 mice when administered in the feed, National Toxicology Programme,
National Technical Information Service, PB 84 - 181734.

Rock G, Figueredo A (1976)
Metabolic changes during platelet preservation.
Transfusion 16 : 571-579

Rock G, Secours VE, Franklin CA, Chu I, Villeneuve DC (1978)
The accumulation of mono 2-ethyl hexyl phthalate (MEHP) during storage of whole blood & plasma.
Transfusion 18 : 553-558

Rock G, Tittley P, Sherring V, Culley C, Wong SC (1981)
Platelet storage : an assessment of the requirements for plasma & oxygen.
Transfusion 21 : 167-177

Rock GA, Farrah G, Rozon G, Smiley RK, Cole R, Villeneuve D, Tittley P (1983)
Analysis of contaminats in Factor VIII preparations administered to patients with hemophilia.
Can. Med. Assoc. J. 128 : 403 - 408

Rock G, Sherring VA, Tittley P (1984a)
Five-day storage of platelet concentrates.
Transfusion 24 : 147-152

Rock G, Tocchi M, Ganz PR, Tackaberry ES (1984b)
Incorporation of plasticizer into red cells during storage.
Transfusion 24 : 493-498

Rock G Swenson SD, Adams A (1985)
Platelet storage in a plasma-free medium.
Transfusion 25 : 551-556

Rock GA, Labow S, Tocchi M (1986)
Distribution of di(2-ethyl hexyl)phthalate and products in blood and blood components.
Environ. Health. Perspect. 65 : 309-316

Rubin RJ, Schiffer CA (1976)
Fate in humans of the plasticiser di(2-ethyl hexyl)phthalate arising from transfusion of platelets stored in vinyl plastic bags.
Transfusion 16 : 330-335

Ryan CJ, Courtney JM, Wood CB, Hood RG, Blumgart LH (1979)
Activated charcoal haemoperfusion via an extracorporeal circuit in the unrestrained and unanaesthetised rat.
Brit. J. Exp. Path., 60 : 400-410

Rzad L, Murphy S, Buchholz DH, Davisson W, Gajewski M, Aster RH (1982)
Storage of platelet concentrate for seven days in PL-732 plastic.
Transfusion 22 : 417

Scott EP, Slichter SJ (1980)
Viability & function of platelet concentrates stored in CPD - adenine (CPDA-1).
Transfusion 20 : 489-497

Shintani H, Tsuji K, Mizumachi S, Oba T, Koshimura E (1984)
The breakdown of phthalates in human serum & plasma & their determination in blood products.
Esei Kagaku (Jpn) 30 : 356-359

Simon TL, Nelson EJ, Carmen R, Murphy S (1983)
Extension of platelet concentrate storage.
Transfusion 23 : 207-212

Slichter SJ, Harker LA (1976)
Preparation & storage of platelet concentrates II. Storage variables influencing platelet viability & function.
Brit. J. Haemat 34 : 403

Sloviter H A (1976)
Physiological & metabolic aspects of freezing erythrocytes with historical notes, in Clinical Uses of Frozen-thawed Red Blood Cells.
Alan R. Liss, New York

Smith A U (1950)
Prevention of haemolysis during freezing & thawing of red blood cells.
Lancet 2 : 910

Snyder EL, Ferri P, Brown R, Gallup P, Roberts S (1985)
Evaluation of flatbed reciprocal motion agitators for resuspension of stored platelet concentrates.
Vox Sang 48 : 269-275

Stern IJ, Carman RA (1980)
Haemolysis in stored blood: Stabilizing effect of phthalate plasticizer.
Proc. Int. Soc. Hematol. Blood Transfus., Montreal. 1980 p.151

Szycher M (1983)
Thrombosis, haemostasis and thrombolysis at prosthetic interfaces.
In: Biocompatible polymers, metals and composites.
Ed: Szycher M, Technomic, Lancaster, Pennsylvania 2-33

Travers M, Courtney JM, Douglas JT, Forbes CD, Lowe GDO, Falkenhagen D, Klinkmann H (1986)
Hemodialysis membranes and the platelet release reaction.
Progress in Artificial Organs - 1985 (eds) Y Nosé, C Kjellstrand, P Ivanovich, ISAO Press, Cleveland pp 1056-1058

Turnbull D, Rodricks JV (1985)
Assessment of possible carcinogenic risk to humans resulting from exposure to di (2-ethyl hexyl) phthalate (DEHP).
J. Am. Coll. Toxicol 2 : 111

Turner JH, Petricciani JC, Crouch ML, Wenger S (1974)
An evaluation of the effects of di ethyl hexyl phthalate (DEHP) on mitotically capable cells in blood packs.
Transfusion 14 : 560-566

United States National Cancer Institute Environ News: EPA bars manufacture of six new plastic compounds. Environmental Protection Agency, Washingotn, D.C. April 28,1980.

Valeri CR, Mercado-Lugo R, Danon D (1965)
Relationship between osmotic fragility & in vivo survival of autologous deglycerolized resuspended red blood cells.
Transfusion 5 : 267-272

Valeri CR, Contreras TJ, Feingold H, Sheibley RH, Jaeger RJ (1973)
Accumulation of di(2-ethylhexyl)phthalate (DEHP) in whole blood platelet concentrates and platelet poor plasma. I. Effect of DEHP on platelet survivial and function.
Environ. Health Perspect. 3 : 103-118

Valeri CR, Feingold H, Marchionni LD (1974)
The relation between response to hypotonic stress & the ^{51}Cr recovery in vivo of preserved platelets.
Transfusion 14 : 331-337

Vessman J, Rietz G (1974)
Determination of di(2-ethyl hexyl) phthalate in human plasma and plasma proteins by electron capture gas chromatography.
J. Chromotogr. biomed. App. 100 : 153 - 163

Vessman J, Rietz G (1978)
Formation of mono (ethyl hexyl)phthalate from di(ethyl hexyl)phthalate in human plasma stored in fractional plasma proteins
Vox Sang. 35 : 75-80

Villeneuve DC, Franklin CA, Chu I, Yagminas A, Marino IA, Ritter L, Ruddick JA (1978)
Toxicity studies on mono 2-ethyhexyl phthalate.
Society of Toxicology, 17 Annu. Meet, San Francisco, Calif. 1978.

Wallace J (1977)
Blood Transfusion for Clinicians.
Churchill Livingstone, Edinburgh London & New York, p8.

Ward JM, Diwan BA, Oshima M, Hu H, Schuller HM Rice JM (1986)
Tumor - initiating and promoting activites of di(2-ethyl hexyl) phthalate in vivo and in vitro.
Environ. Health Perspect. 65 : 279

Warren JR, Lalwang ND, Reddy JK (1982)
Environmental Health Perspectives, 45, 34

Weed RI (1970)
The importance of erythrocyte deformability.
Am. J. Med. 49 : 147

Weiss HJ (1975)
Platelet physiology & abnormalities of platelet function.
New Eng. J. Med. 293 : 531-541

Wessler S, Gitel S N (1979)
Heparin : new concepts relevant to clinical use.
Blood 53 : 525-544

White JG, Hagert K, Nipper JHJ, Rao GHR (1980)
Functional platelets after storage in vitro for 15-21 days.
Am. J. Pathol. 101 : 613-634

WHO (1992)
Diethylhexyl phthalate : Environmental health criteria 131
In: International Programme on Chemical Safety
Ed: World Health Organisation, Geneva

Wilson RH, McCormick WE (1954)
Ind. Med & Surg. 23, 479

Wolkowski-Tyl R, Jones-Price C, Marr MC, Kimmel CA (1983)
Teratologic evaluation of diethyl hexyl phthalate in Fischer F344 rats.
Teratology 27 : 85a

Woodward KN (1988)
Phthalate Esters : Toxicity & Metabolism, Vol 1.
ed. Boca Raton, CRC Press