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(54) **AMELIORATION DU STOCKAGE ET DE LA CONSERVATION DE PRODUITS SANGUINS**

(54) **IMPROVED STORAGE AND MAINTENANCE OF BLOOD PRODUCTS**

(57) On améliore la conservation de la membrane cellulaire des globules rouges et des concentrés de plaquettes en ajoutant entre 1 mM et 10 mM de L-carnitine et de dérivés de celle-ci. Cette amélioration permet d'étendre la période de viabilité de globules rouges et de concentrations de plaquettes conditionnés au-delà des périodes admises jusqu'à présent. En outre, les matières traitées de la sorte présentent une demi-vie de circulation étendue lorsqu'elles sont transfusées à un patient. L'amélioration de la conservation de la membrane résultant de ce procédé permet d'irradier des récipients hermétiquement fermés de produits sanguins et de rendre sensiblement stériles ces derniers tout en détruisant les leucocytes qu'ils contiennent.

(57) Cell membrane maintenance of red blood cells and platelet concentrates is improved by the addition of 1 mM-10 mM L-carnitine and derivatives. This improvement allows extension of the period of viability of packed red blood cells and platelet concentrations beyond current periods. Additionally, the materials so treated exhibit extended circulation half-life upon transfusion to a patient. Improvements in membrane maintenance achieved by this method permit irradiation of sealed containers of blood products so as to substantially sterilize same and destroy leukocytes in the same.

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(54) Title: IMPROVED STORAGE AND MAINTENANCE OF BLOOD PRODUCTS

(57) Abstract

Cell membrane maintenance of red blood cells and platelet concentrates is improved by the addition of 1 mM–10 mM L–carnitine and derivatives. This improvement allows extension of the period of viability of packed red blood cells and platelet concentrations beyond current periods. Additionally, the materials so treated exhibit extended circulation half–life upon transfusion to a patient. Improvements in membrane maintenance achieved by this method permit irradiation of sealed containers of blood products so as to substantially sterilize same and destroy leukocytes in the same.

IMPROVED STORAGE AND MAINTENANCE OF BLOOD PRODUCTS

Field of the Invention

This invention pertains to a method of improving the storage stability, including resistance to hemolysis and improved viability, of blood products including packed red blood cells (RBCs), platelets and the like. Specifically, a method for extending the viability of these products, as well as their resistance to membrane damaging agents such as radiation, is provided by storing the products in a suspension including an effective amount of L-carnitine or alkanoyl L-carnitines.

BACKGROUND OF THE INVENTION

Concern has been steadily growing over both the national and worldwide blood supplies. Both the integrity and reliability of existing supplies, and the ability to build larger stocks over time, has been brought into question. One reason for this is the relatively short period of storage stability of blood products. Currently, packed RBCs (red blood cell concentrates, or RCC), the dominant form of blood product for transfusions and the like, are limited to a 42-day storage period. After that time, ATP levels fall substantially, coupled with a significant loss of pH, strongly indicating a lack of viability, or, if viable, an extremely short circulation life upon infusion. *In vivo* whole blood is not stored for substantial periods. For platelets, the current storage period is even shorter, with the standard being 5-days at 22°C. The difference in storage stability of platelet concentrations (PC) as opposed to RBC, is

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due to ongoing metabolic reactions in platelets, due in part to the presence of mitochondria in PC, and their absence in RBCs. While both blood products show a drop in ATP, coupled with a drop in pH, over time, accompanied by the production of lactic acid, the presence of mitochondria in PC is likely to exacerbate the problem, due to glycolysis.

Simultaneously, concerns over the reliability and integrity of the blood supply have been raised. In particular, contamination of the blood supply with bacteria, or other microbiological agents or viruses, has been detected repeatedly. Such a situation is even more severe in countries with less sophisticated collection and storage methods. While agents may be added to collected products to reduce contamination, these are not desirable, given the need to transfuse the products back into recipient patients. One desirable alternative is irradiation treatment of the products, after packaging, typically in elasticized vinyl plastic containers. Such irradiation treatment would aggravate RBC and PC storage, resulting in a diminished function of these cells.

Additionally, a small but growing portion of the blood receiving population is at risk of a generally fatal condition known as Transfusion Associated Graft Versus Host Disease (TAGVHD), which is due to the presence of viable allogenic leukocytes. This syndrome is typically associated with immunosuppressed patients, such as cancer and bone marrow transplant patients, but can also occur in immunocompetent

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persons in the setting of restricted Human Leukocyte Locus A (HLA) polymorphism in the population.

Substantial attention has been devoted to finding methods to extend storage stability. One such method, for extending the storage lifetime of PCs, is recited in U.S. Patent 5,466,573. This patent is directed to providing PC preparations with acetate ion sources, which act both as a substrate for oxidative phosphorylation and as a buffer to counteract pH decrease due to lactic acid production. Such a method does not act directly on the problem of hemolysis, and membrane breakdown. An alternative method is disclosed in the commonly assigned U.S. Patent 5,496,821. In this patent, whole blood is stored in a preparation including L-carnitine (LC) or alkanoyl derivatives thereof. The patent does not describe, however, the effects on blood products such as PC or RBC suspensions, and relies to at least some extent on the impact of LC on plasma characteristics.

As noted above, contamination of the blood supply with microbiological agents or viruses is another problem to be addressed by the medical community. One method of sterilizing the product and improving reliability with respect to contamination, is to irradiate the blood product. In general, gamma irradiation values of about 25 centigray (cG), irradiating the product after it is sealed in a plastic, glass or other container, is desirable. Regrettably, irradiation induces cell membrane lesions, with hemolysis in RBCs. Irradiation of blood

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products, including whole blood, packed RBCs and PCs continue to pose problems.

Accordingly, it remains an object of those of skill in the art to provide a method to extend the period of viability, and the circulation half-life of RBCs and PCs upon transfusion, beyond the current maximums. Additionally, it remains a goal of those of skill in the art to find a way by which blood products, including whole blood, packed RBCs and PCs can be sterilized by irradiation, without substantial membrane damage and lesions, and hemolysis.

SUMMARY OF THE INVENTION

The Applicant has discovered, through extended research, that the membrane damage experienced by RBCs and PCs upon storage, or in the face of irradiation, can be substantially delayed and suppressed, by suspending the blood product in a conventional preservation solution, such as AS-3, where the preservation solution further includes L-carnitine or an alkanoyl derivative thereof, in a concentration of 0.25-50 mM or more. Applicant's discovery lies in the recognition that most of the decomposition or blood products, conventionally associated with decreases in ATP levels and pH, can be in fact traced to membrane damage and hemolysis. Membrane maintenance and repair may be effected by lipid reacylation, effected, in part, through L-carnitine, the irreversible uptake of which in RBC and similar blood products has been established through the inventive research.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Lifespan values after infusion of 42 days stored RBC as related to donor and to control and LC stored. The arrows indicate mean ± SD of control and LC stored RBC, respectively. On top of the graph, the exact calculated p value is also shown.

Figure 2. Red cell carnitine content at different weeks of blood preservation. Carnitine was assayed as described in Materials and Methods. Values are the average of three experiments done in duplicate. The variation between experiments was not more than 7%. Open symbols, RBC stored with AS-3 alone; closed symbols, RBC stored in AS-3 supplemented with LC (5 mM).

DETAILED DESCRIPTION OF THE INVENTION

This invention employs L-carnitine, and its alkanoyl derivatives, as an agent supporting cell membrane maintenance and repair, and suppression of hemolysis, in blood products. Alkanoyl L-carnitines includes acetyl, butyryl, isobutyryl, valeryl, isovaleryl and particularly propionyl L-carnitine. Herein, reference is made to this family, generically, as LC, and exemplification is in terms of L-carnitine. The described alkanoyl L-carnitines, and their pharmacologically acceptable salts, however, may be used in place of L-carnitine.

The addition of LC to blood products, including RBCs and PCs, requires LC to be present in an amount effective to permit membrane maintenance, repair and hemolysis suppression. The research

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undertaken, including the examples set forth below, has demonstrated a minimum effective range for the products of most donors of about 0.25 mM-0.5 mM. The upper limit is more practical than physiological. Concentration as high as 50 mM or greater are easily tolerated. Values that are consistent with toxicological and osmological concerns are acceptable. Preferred ranges are 1-30 mM. A range of 1-10 mM or more is suitable with values between 4-6 mM making a marked difference. The effects of this invention, including the prolongation of viability, and the extension of circulation half-life upon transfusion, may be highly donor dependent. Accordingly, generally speaking, an effective concentration of LC is 0.5-50 mM. However, the ordinary artisan in the field may be required to extend that range, in either direction, depending on the particularities of the donor. Such extensions do not require inventive effort.

LC is consistent with conventional support solutions (stabilizing solutions), which are typically prepared to provide a buffering effect. Commonly employed solutions include ACED (citric acid-sodium citrate-dextrose), CPD (citrate-phosphate-dextrose) and modifications thereof, including CPD2/A-3, and related compositions. Typically, the composition includes a carbohydrate, such as glucose or mannitol, at least one phosphate salt, a citrate, and other balancing salts. LC is conventionally soluble and may be added to these compositions freely within the required range.

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Suitable solutions, are described in U.S. Patent 5,496,821, incorporated herein-by-reference. However, support solutions other than those conventionally used can be employed, including artificial plasma and other physiologically acceptable solutions provided they comprise LC in accordance with the invention. The important component of the support solution is LC.

The ability of LC, when included in the suspension of blood products such as RBC and PCs, to extend the viable time and therefore shelf length, and the circulation period upon transfusion into the receiving individual, is exemplified below by *in vitro* and *in vivo* experimentation. The experimentation employs LC, but other alkanoyl L-carnitines can be employed. Of particular significance is the demonstration, below, that the improved performance is obtained through improved maintenance (including repair) of the cell membrane, and suppression of hemolysis.

MATERIALS AND METHODS

Study design I:

Evaluation of *in vivo* and *in vitro* quality of RBC stored with and without LC

Subjects. The subject population was male or female research subjects between the ages of 18 to 65 years with no known mental or physical disability and taking no drugs that might affect RBC viability. Individuals were recruited who fulfilled the conventional allogenic donor criteria as listed in the Code of Federal Register, Chapter 2, the

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Standards of the American Association of Blood Banks, and the Blood Services Directives of the American National Red Cross. The study was approved by the Institutional Review Board of the Medical College of Hampton Roads and the subjects gave informed consent prior to participation in the study.

Each donor donated on two different occasions separated by 72 days, and was randomized to either the test or control arm on the first donation.

Blood storage system. Standard CP2D/AS-3 system (Miles, Inc.)

Polyvinyl chloride (PVC) plastic bags with diethyl-(n)hexylphthalate (DEHP) as plasticizer were used. For each test unit, 245 mg LC (in 1.1 mL pure, pyrogen-free solution in a sterilized glass bottle) was added to the container holding the AS-3 additive solution to give a final concentration of 5 mM. For the control units, 1.1 mL 0.9% NaCl was added to the AS-3 solution using the same conditions. Addition of LC or saline to the bags was performed by injecting through a sampling site coupler with a syringe. This was done in a laminar flow hood under UV light.

Donation & Processing. Standard phlebotomy and blood-drawing methods were used with collection of approximately 450±50 mL whole blood. The whole blood unit was held between 4-8 hours at room temperature before processing. The unit was centrifuged using standard conditions and, after centrifugation, the supernatant plasma was

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expressed off, and the sedimented packed RBC resuspended either in the standard AS-3 solution (control) or the L-carnitine-containing AS-3 solution (test). The suspended RBC units were stored at 4° C for 42 days.

***In Vitro* Measurements:** Measurements performed on pre- (0 day) and post- (42-day) samples included RBC ATP levels; total and supernatant hemoglobin; hematocrit (Hct); RBC, WBC, and platelet counts; RBC osmotic fragility; RBC morphology; lactate and glucose levels; supernatant potassium levels. These were performed using standard procedures as described previously, Heaton et al., Vox Sang 57:37-42 (1989).

***In vivo* Post-transfusion Measurements.** After 42 days of storage, a sample was withdrawn and the stored cells labeled with Cr using standard methods. At the same time, to determine RBC mass, a fresh sample was collected from the donor for RBC labeling with 99 Tc. After labeling, 15 μCi 51 Cr-labeled stored cells and 15 μCi 99 Tc-labeled fresh cells were mixed and simultaneously infused. Blood samples (5 mL) were taken after the infusion at various time intervals for up to 35 days to calculate 24-hour % recovery and survival. The 24-hour % recovery was determined using either the single label method where log-linear regression of the radioactivity levels of samples taken at 5, 7.5, 10, and 15 min. was used to determine 0 time level, or by the double

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label method using donor RBC mass as determined by the 99 Tc measurement.

Circulating lifespan of the transfused surviving Cr-labeled RBC was determined by samples taken at 24 hours and then twice weekly for up to 5 weeks. The radioactivity levels were corrected for a constant 1% elution per day. The data were fitted to a linear function with post-transfusion days as independent variable (x-axis) and the corrected Cr counts as dependent variable (y-axis). The lifespan of the RBC was then taken as the intersection of the fitted line with the x-axis.

Statistical analysis. Paired t-test or routine non-parametric statistical analysis was performed on data from the *in vivo* and *in vitro* testing of the units to determine if there were any statistically significant differences (1-tail) in the means between the test and control units. Statistical significance was considered at a p value less than 0.05.

Study design II

Erythrocytes LC uptake and lipid reacylation studies with storage up to 42 days

Chemicals. Essentially fatty acid-free bovine serum albumin (BSA) was obtained from SIGMA Chemical Company, St. Louis, Mo. (USA).

[1-^{14}C]Palmitic acid (58 Ci/mol) was obtained from New England Nuclear Corporation, Boston, Mass. (USA). Thin-layer plates, Whatman LK6 (silica gel) (20x20 cm) with a pre-absorbent layer were obtained from Carlo Erba, Milan (Italy). All other compounds used were reagent grade.

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***Red cell carnitine* assay.** Blood sample was withdrawn from the stored RCC unit and washed once with 4 vol. of cold 0.9% NaCl. RBC were then resuspended in 0.9% NaCl at a final hematocrit of 50%, and deproteinized with perchloric acid as described, Cooper et al., Biochem. Biophys. Acta 959: 100-105 (1988). Aliquots of the final extract were analyzed for free LC content according to the radiochemical assay of Pace et al., Clin. Chem. 24: 32-35 (1978).

Results

Study I

Pre-storage AS-3 RCC unit characteristics

The properties of the AS-3 RCC products were as expected after the processing of the whole blood units. No significant difference between test and control units in the characteristics of the AS-3 RBC unit were observed as measured by unit volume, Hct, and WBC content, and *in vitro* RBC properties such as ATP levels, supernatant hemoglobin and potassium levels, osmotic fragility (Table I).

Post-42 day storage RBC characteristics

Metabolic. The amounts of glucose consumed and lactate produced during 42 days of storage were similar for test and control units (Table 2). As expected, an inverse high correlation was found between these two parameters of glycolysis (r=0.76). However, less hemolysis and higher ATP levels were found for carnitine-stored RBC units as

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compared to control. As illustrated in Figure 1, this higher ATP level was observed in all but one pair ($p<0.01$).

Membrane. Percent hemolysis at the end of storage levels was less with the L-carnitine units, as shown in Figure 2. On the other hand, no significant differences were found with regard to supernatant potassium levels, osmotic shock response, and morphology score which were within expected range at the end of 42 days of storage.

Post transfusion viability. The mean 24-hour % recovery for the control units was similar to what previously has been found. However, mean % recoveries for the carnitine-stored red cells were higher than the control-stored cells ($p<0.05$). In addition, the mean circulating lifespan of the infused stored red cells was also higher for the L-carnitine-stored cells (Figure 1). The donors' RBC mass as determined on the two occasions were highly similar ($r=0.98$) and statistically not different.

Correlation studies. As expected, 24-hour % recovery showed significant correlations with ATP levels ($r=0.63$) and other measurements of RBC membrane integrity such as hemolysis ($r=0.57$), osmotic fragility ($r=0.71$), and morphology score ($r=0.59$). Percent hemolysis correlated highly with ATP levels ($r=0.83$) and also with the WBC content of the RBC units ($r=0.83$). The RBC circulating lifespan showed no significant correlations with any *in vitro* parameter.

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Study II

Carnitine uptake in stored RBC

RBC stored in AS-3 medium alone did not show any significant loss of the LC content throughout the storage (Figure 2). This is in agreement with findings by Cooper et al., *supra* showing that human red cell LC does not freely exchange with either plasma or isoosmotic buffer. When red cells were stored in AS-3 supplemented with LC, higher amounts of intracellular LC than AS-3 alone were detected (Figure 2). LC content increased linearly during times of storage, reaching a 4 fold increase at 42 days.

Discussion

In vitro and *in vivo* testing of RBC units at the end of 42 days of storage demonstrated significant differences between carnitine-stored RBC as compared to control-stored RBC. Various *in vitro* RBC properties reflective of metabolic and membrane integrity such as ATP and % hemolysis, as well as direct measure of cell viability (24-hour % recovery and circulating lifespan) were significantly superior for carnitine-stored RBC. A prolongation of the mean lifespan of the surviving RBC circulating at 24-hours after infusion is of interest. This finding may be related to the irreversible uptake of LC during storage an unprecedented and unexpected discovery. The values obtained in the control studies for various RBC properties were as expected and not different from previous studies. At the time of blood collection, no significant

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differences in unit or RBC characteristics between test and control were found. As illustrated in Figures 1-3, RBC ATP levels, % hemolysis, and circulating lifespan were strongly donor-related and since the study was a randomly paired design with five test and five control studies performed on both the first and second occasions, it is unlikely that the observed differences could be due to chance or to any faulty study design. It is, therefore, apparent that the observed differences found in this study were caused by the addition of L-carnitine to the test units. The possibility that the increased lifespan reflects decreased elution of Cr cannot be excluded, but is not consistent with the improved *in vitro* measures of the stored red cells that has been found to correlate with *in vivo* viability.

Several investigations have found that L-carnitine and its acyl-esters have a cytoprotective/membrane stabilizing effect on various cells including red cells. See, e.g., Snyder et al., Arch. Biochem. Biophys. 276: 132-138 (1990). In this study it was found that L-carnitine was irreversibly taken up by the RBC during storage. Although the nature of this process is not entirely clear, one would exclude the participation of a specific carrier for the L-carnitine uptake. To our knowledge, the only known L-carnitine carrier operates in cellular systems where the intracellular concentration of L-carnitine is several fold higher than that normally present in the extracellular environment. Red cell L-carnitine concentration is similar to that of the plasma. Thus,

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irrespective of the low temperature, APT depletion, and other possible metabolic changes occurring during the storage, when red cells are stored in a medium containing relatively high amounts of exogenous L-carnitine, a unidirectional uptake of L-carnitine by the cells seems to be established. Nothing in the art appears to predict this.

The nature of the action of L-carnitine on stored RBC could be viewed either as a biophysical and/or metabolic intervention on the membrane compartment. Post-transfusion survival of stored red cells is related to the integrity of membrane function as suggested by the significant correlation between the *in vivo* viability of reinfused red cell and its surface-to-volume ratio measures. A major contributor to RBC membrane structure and function is represented by the cytoskeleton network, Marchesi, Ann. Rev. Cell Biol. 1: 531-536 (1985), a supramolecular protein organization lying beneath the inner hemileaflet of RBC membrane. Wolfe et al in a survey study on the composition and function of cytoskeletal membrane protein of stored red cells found that the only relevant change was a decreased capability of spectrin to associate with actin either in the presence or absence of protein 4.1. Wolfe et al., J. Clin. Invest. 78: 1681-1686 (1986). We have shown that L-carnitine affect RBC membrane deformability of protein 4.1 containing resealed ghosts subjected to increased shear stress. Arduini et al., Life Sci. 47: 2395-2400 (1990). Thus, L-carnitine may exert a stabilizing effect of the membrane through a spectrin interaction with

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one or more cytoskeletal components. A recent electron paramagnetic resonance study of Butterfield and Rangachari, Life Sci. 52: 297-303 (1992), on the red cell spectrin-actin interaction showed that L-carnitine significantly reduced the segmental motion of spin-labeled sites on spectrin.

In addition to a potential biophysical action described above, the improvements observed in the LC-stored red cells may also be the result of a favorable metabolic process. Normally, the deacylation-reacylation cycle of membrane phospholipids requires ATP or generation of acyl-CoA. The acyl moiety of acyl-CoA is then transferred into lysophospholipids by lysophospholipid acyl-CoA transferase. In addition, during an oxidative challenge, the membrane repair process of RBC phospholipids follows the same metabolic pathway. Recent findings have shown that CPT affect the reacylation process of membrane phospholipids in red cells and neuronal cells by modulating the size of the acyl-CoA pool between the activation step of the fatty acid and its transfer into lysophospholipids. Arduini et al., J. Biol. Chem. 267: 12673-12681 (1992). In addition, pulse-chase and ATP depletion studies have demonstrated that the red cell acylcarnitine pool serves as a reservoir of activated acyl groups at no cost of ATP. Arduini et al., Biochem. Biophys. Res. Comm :187: 353-358 (1994).

The higher 24-hour % recovery and circulating lifespan represents an improvement of approximately 15% in terms of potency

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(amount of transfused circulating RBC available to the recipient times average lifespan). This increase in potency could translate clinically into a reduction in transfusion requirements in chronically transfused patients such as in thalassemics or in patients with bone marrow failure. Alternatively, it may be possible to extend the shelf-life of liquid stored RBCs.

Our findings suggest that the presence of L-carnitine in the preservation. medium during RBC storage may have a sparing action on the ATP pool used by the reacylation of phospholipids for membrane repair. This favorable metabolic process, associated with a possible beneficial biophysical action, may thus explain the reduced hemolysis, higher ATP levels, and the improved *in vivo* recovery and survival of the LC-stored red cells.

IRRADIATION

The problem of contamination of blood products, including whole blood, RBC, PC and the like with bacteria or other microbiological agents or viruses, can be reduced by substantial amount by irradiation. Levels of irradiation necessary for sterilization, and substantially 100% mortality of the aforesaid contaminating agents, have been widely explored.

Additionally, more importantly, leukocytes may be destroyed by similar irradiation. A variety of types of irradiation can be used, including gamma radiation (Cobalt GG, Van de Graf acceleration), UV

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irradiation, red light irradiation, etc. A close equivalent to about 20-50 cG gamma irradiation is sufficient.

Worldwide, between 60 and 80 million units of whole blood are collected annually and used in the transfusion support of a variety of patient populations. In the underdeveloped countries collection rates per 1000 population are lower and most blood transfusions are given in the treatment of obstetrical and pediatric cases, particularly malaria associated anemia. In the developed countries, collection rates per 1000 population are 50-10 times higher and most transfusions are given in surgery (50%) or in the treatment of patients with cancer associated anemia, bone marrow transplantation, non-malignant gastrointestinal bleeding (Figure 1). There are many potential adverse effects associated with the transfusion of allogenic blood. One particular complication adversely associated with blood transfusion is the rare and usually fatal entity known as Transfusion associated graft versus host disease (TA-GVHD), a complication mediated by viable allogenic immunocytes.

TA-GVHD disease is a rare complication of blood transfusion potentially seen in two types of blood transfusion recipient patient populations. TA-GVHD has a mortality approaching 100% and prevention is the only effective approach at this time. First, in immunocompromised patients, such as patients after bone marrow or other organ transplantation, Hodgkins disease or hereditary deficiencies of the immune system. Second, in nonimmunocompromised patients,

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when HLA similarity exists between blood donor and blood recipient. This is most often seen in directed donations from close relatives or in populations of more limited HLA polymorphism such as in Japan and Israel. On account of this, it is universal practice to irradiate cellular blood products with gamma irradiation to a mid-plane dose of approximately 25 centigray (cG) in order to destroy the replicating ability of viable immunocytes. It should be noted that TA-GVHD is associated with cellular products which are fresh, i.e. generally less than 15 days. However, "aging" of blood is not as yet an accepted practice in preventing this complication. Although the immunocytes are part of the allogenic leukocyte population, the degree of leukodepletion currently achieved with third generation filters is not considered currently adequate to prevent this complication. Thus, gamma irradiation at this time remains the only accepted prophylactic intervention.

The difficulty with gamma irradiation of red cells in particular is the potential to damage the cell membrane. It is clear that irradiation to this dose produces a loss in potency of approximately 7-8% as measured *in vitro* by a reduction in red cell ATP, increased hemolysis, and increased supernatant potassium. These changes are consistent with a membrane damage effect. These *in vitro* changes are associated with a reduction in the 24 hour recovery of gamma irradiation red blood

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cells. With regard to platelet products, some loss in viability has also been reported.

Considerable evidence indicates that gamma irradiation exerts its effects by generating activated oxygen species, such as singlet oxygen, hydroxy, radical, and superoxide anion. These species induce intracellular damage to DNA, thus prevent cell replication, a prerequisite to TA-GVHD. However, these same oxygen species may oxidize membrane lipids on the red cell and possibly platelet membrane, inducing a membrane lesion which reduces the quality (potency) of the cellular product.

L-carnitine is known to play a key role in the transportation of long chain fatty acids across the mitochondrial membrane. Hereditary disorders in which there is a failure of the carnitine system to transport long chain fatty acids results in significant impairment in skeletal muscle function. Recently, there has been increasing interest in the role of L-carnitine in red cell membrane. What has been surprising, however, is that red cells lack mitochondria, and thus, considerable curiosity surrounding the presence of L-carnitine and carnitine palmitoyl transferase, an enzyme involved in reversible acylation of L-carnitine. It was at first unclear as to the role which these might play within red cell. The red cell may be subjected to oxidant stress throughout it's long life cycle *in vivo*, and repair of oxidized membrane

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lipids involving L-carnitine could be important for the normal survival of red cells.

Early increase in acylated carnitine during a time of increased ATP availability may function as a reservoir of activated fatty acids, which can subsequently be used in a repair mechanism for damaged oxidized membranes lipids. Such an explanation would well explain the reduced hemolysis observed during the *in vitro* storage of red blood cells supra and in addition, would explain the improvement observed with *in vivo* recovery and survival. The net effect of L-carnitine addition is an approximate 17% increase in potency.

Accordingly, L-carnitine or the aforesaid alkanoyl L-carnitines may be used to abrogate or prevent membrane lesions induced by irradiation. This would occur through the ability of carnitine stored in red cells to repair oxidized membrane lipids *in vitro*.

To limit blood products (RCC, PC and the like) susceptibility to membrane lesions and hemolysis, the blood product may be first suspended in a solution including L-carnitine or one of the aforesaid alkanoyl L-carnitines in an amount of 0.25 mM - 50 mM. Cell membrane maintenance and suppression of hemolysis is achieved to a sufficient degree that the sealed product can be irradiated for the purposes of sterilization, and subsequently may enjoy an extended shelf life and circulation half-life after transfusion. Viability on the order of current viabilities can be achieved, with materials more nearly certain

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to be sterile and unlikely to introduce TA-GVHD, due to irradiation after sealing the blood product suspension. It is to be emphasized that the term blood product, in this connection, is to be interpreted broadly, to include whole blood, blood plasma, RCCs, PCs, mixtures and the like.

To confirm that the addition of L-carnitine to red blood cell compositions to be gamma-irradiated to sterilize it or destroy immunocytes therein followed by storage improves red blood cell survival, the following tests were conducted.

Materials and Methods

Blood storage system. Standard quadruple blood storage bag CP2D/AS-1 systems (Baxter, Inc.) were used. For each test unit, 245 mg L carnitine inner salt (LC) dissolved in 1.1 mL pure, pyrogen-free saline solution were added to the bag holding the AS-1 additive solution to give a final 5 mM concentration. For the control units, 1.1 mL saline solution was added to the AS-1 solution under the same conditions.

Donation and Processing. Standard phlebotomy and blood-drawing methods were used with collection of approximately 450 ± 50 mL whole blood. The whole blood unit was held between 4-8 hours at room temperature before processing. The unit was centrifuged using standard conditions (see: Heaton W.A. et al; Vox sanguinis, 1989, 57: 37-42) and, after centrifugation, the supernatant plasma was poured off, and the sedimented packed red blood cells (RBC) resuspended either in the

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standard AS-1 solution (control) or the LC containing AS-1 solution (test).

Storage Conditions and Gamma Irradiation of RBC Units. The suspended RBC units (control and test) were stored at 1-6° C for 14 days, and then gamma irradiated to a dose of 25 cG. Subsequently, RBC units were stored at 1-6° C until 42 days.

In Vitro Measurements. Measurements performed on pre- (0 day) and post- (42 day) sample included supernatant hemoglobin and mean corpuscular volume (MCV). These were performed using standard procedures as described in Heaton et al., supra.

Statistical Analysis. The sample size is based on previous data regarding variances in supernatant hemoglobin. Thus a total number of 94 RBC units were drawn, 47 in each arm (control and test). Analysis of the data distribution for plasma hemoglobin showed significant skew (>2) and kurtosis (8-10). Therefore, the data were analyzed using non-parametric tests for medians on a software program (Epistat, Richardson, TX).

Results

After the processing of the whole-blood units, the properties of the RBC units before storage were as expected. No significant difference between control and LC-containing RBC units were observed in supernatant hemoglobin and MCH. (Table A). At the end of the storage (42 days), the gamma-irradiated RBC presented statistically significant differences between the control and LC-containing units (see p values in

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table A). In particular, a lower hemolysis was observed in the test compared to control RBC units. The MCV value of RBC unit containing LC was lower than that of the control.

The above test demonstrates that the damages associated with gamma-irradiation of red blood cell products followed by storage are prevented by addition of L-carnitine to the red blood cell product.

TABLE A

	DAY 0			DAY 42		
	CONTROL	L-CARNITINE	p	CONTROL	L-CARNITINE	p
MCV (fL)	91.2	90.7	0.46	100.2	97.5	0.006
Plasma Hb (mg/dL)	11.8	11.5	0.58	495	382	0.05

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The present invention has been disclosed in both generic terms and by reference to specific examples. Variations will occur to those of ordinary skill in the art without the exercise of inventive faculty. In particular, alternate stabilizing compositions, blood products, preservatives, inhibitors and the like may be modified, without the exercise of inventive skill. Additionally, specific levels, viability periods and circulation half-lives will vary from donor to donor, and recipient and recipient. Such variations remain within the scope of the invention, unless specifically excluded by the recitations of the claims set forth below.

Table 1. RBC *Pre-Storage Characteristics of the Red Cell concentrates*

	Control	*Test (L-Carnitine)*
Unit Void (mL)	305±38	295±41
Uni: He: (Jo)	60±3	60±3
Unit WBC (x 10^9)	2.6±1.1	2.4±1.1
ATP (µmol/g Hb)	4.6±0.2	4.3±0.4
Supernatant Hb (mg/dL)	34±15	26±8
Supernatant K+ (mEq/L)	23±0.2	2.2±0.3
Osmotic Fragility (%)	50±4	49±4

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Table 2. *Post-Storage (42 days) Characteristics of the Red Cell concentrates*

	Control	*Test (L-Carnitine)*
In Vitro parameters		
Glucose (mg/dL)	208±33	193±40
Lactate (mg/dL)	201±27	199+37
pH	6.33±0.03	6.32±0.04
ATP (μmol/g Hb)	3.01±0.42	3.24±0.38*
Hemolysis (%)	0.47±0.41	0.30±0.22*
Supernatant K + (mEq/L)	61±4	60±3
Osmotic Fragility (%)	51±3	50±4
Morphological Score	69±8	68±15
In vivo Parameters		
24H % Recovery (single label)	81.1±6.2	84.0±4.4
24H % Recovery (double label)	80.1±6.0	83.9±5.0*
RBC Mass (mL)	1634±510	159 1±534
Survival (days)	85.9±14.3	96.1±11.2*

* (p< 0.05)

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1. A method of improving membrane maintenance and suppressing hemolysis on storage of platelet concentrate (PC), comprising suspending said PC in a support solution comprising a carnitine product selected from the group consisting of L-carnitine, alkanoyl L-carnitines or the pharmacologically acceptable salts thereof and mixtures thereof, in an amount effective to improve the ability of said PC to maintain the membrane of said platelets, and to thereby suppress hemolysis, as compared with an identical support solution lacking said L-carnitine, alkanoyl L-carnitines or the pharmacologically acceptable salts thereof and mixtures thereof.

2. The method of claim 1, wherein said L-carnitine, alkanoyl L-carnitines and mixtures thereof is present in a range of 0.25-50mM.

3. The method of claim 2, wherein said L-carnitine, alkanoyl L-carnitines and mixtures thereof is present in an amount of 1-20mM.

4. A sealed container of PC in a stabilizing solution, said stabilizing solution comprising L-carnitine, alkanoyl L-carnitines and mixtures thereof in an amount of 0.25-50 mM.

5. The sealed container of claim 4, wherein said container, subsequent to sealing, has been irradiated to substantially sterilize it and destroy leukocytes therein.

6. A sealed container of RBC in a support solution, said support solution comprising L-carnitine, alkanoyl L-carnitines or the pharmacologically acceptable salts thereof and mixtures thereof in an amount of 0.25-50 mM, wherein subsequent to sealing, said container has been irradiated so as to substantially sterilize it and destroy leukocytes therein.

7. A method of substantially sterilizing blood products selected from the group consisting of whole blood, plasma, RBC, PC and mixtures thereof, comprising suspending said blood product in a support solution, said solution comprising LC in an amount of 0.25-50 mM, and irradiating said blood product after sealing said product within a container, so as to substantially sterilize said blood product.

8. A method of treating blood products selected from the group consisting of whole blood, plasma, RBC, PC and mixtures thereof to suppress leukocytes therein, comprising irradiating said blood in a support solution comprising LC in an amount of 0.25-50 mM, so as to substantially destroy leukocytes in said sample while suppressing damage to membranes of said blood products.

9. A method of improving membrane maintenance and suppressing hemolysis on storage of a red blood cell (RBC) or platelet concentrate (PC) following irradiation of the same for the purpose of destroying immunocytes therein, comprising suspending said

RBC or PC in a support solution, said support solution comprising a carnitine product selected from the group consisting of L-carnitine, alkanoyl L-carnitines and mixtures thereof, in an amount effective to improve the ability of said RBC or PC to maintain the membrane of said RBC or platelets, and to thereby suppress hemolysis as compared with identical support solution lacking said carnitine, alkanoyl L-carnitine, and mixtures thereof.

10. The method of claims 1, 2, 3, 7, 8 and 9, wherein the alkanoyl L-carnitine is selected from the group comprising acetyl, propionyl, butyryl, isobutyryl, valeryl and isovaleryl L-carnitine.

11. The sealed contained of claims 4-6, wherein the alkanoyl L-carnitine is selected from the group comprising acetyl, propionyl, butyryl, isobutyryl, valeryl and isovaleryl L-carnitine.

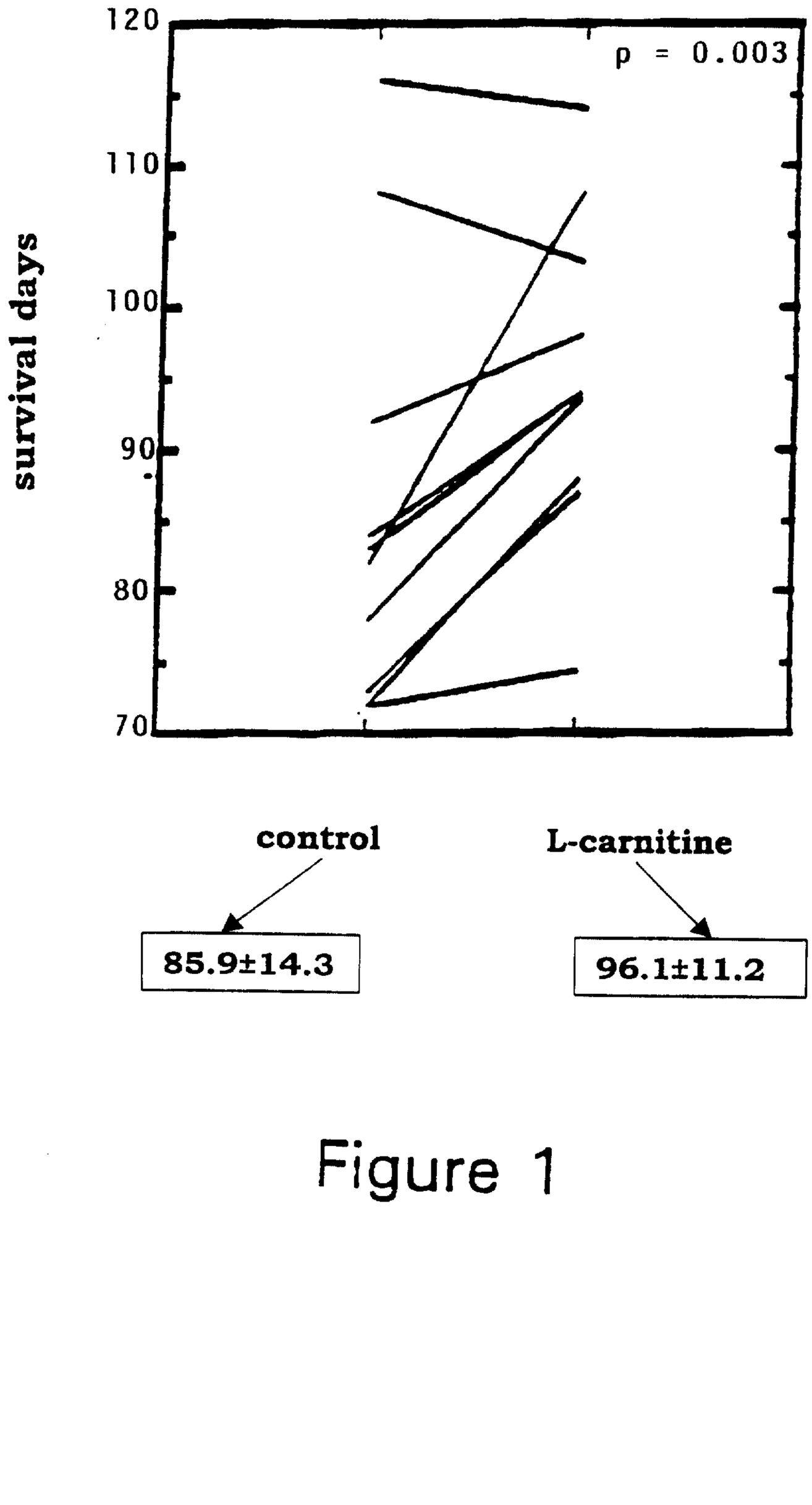
120
110
100
90
80
70
survival days
p = 0.003
control
L-carnitine
85.9±14.3
96.1±11.2

Figure 1

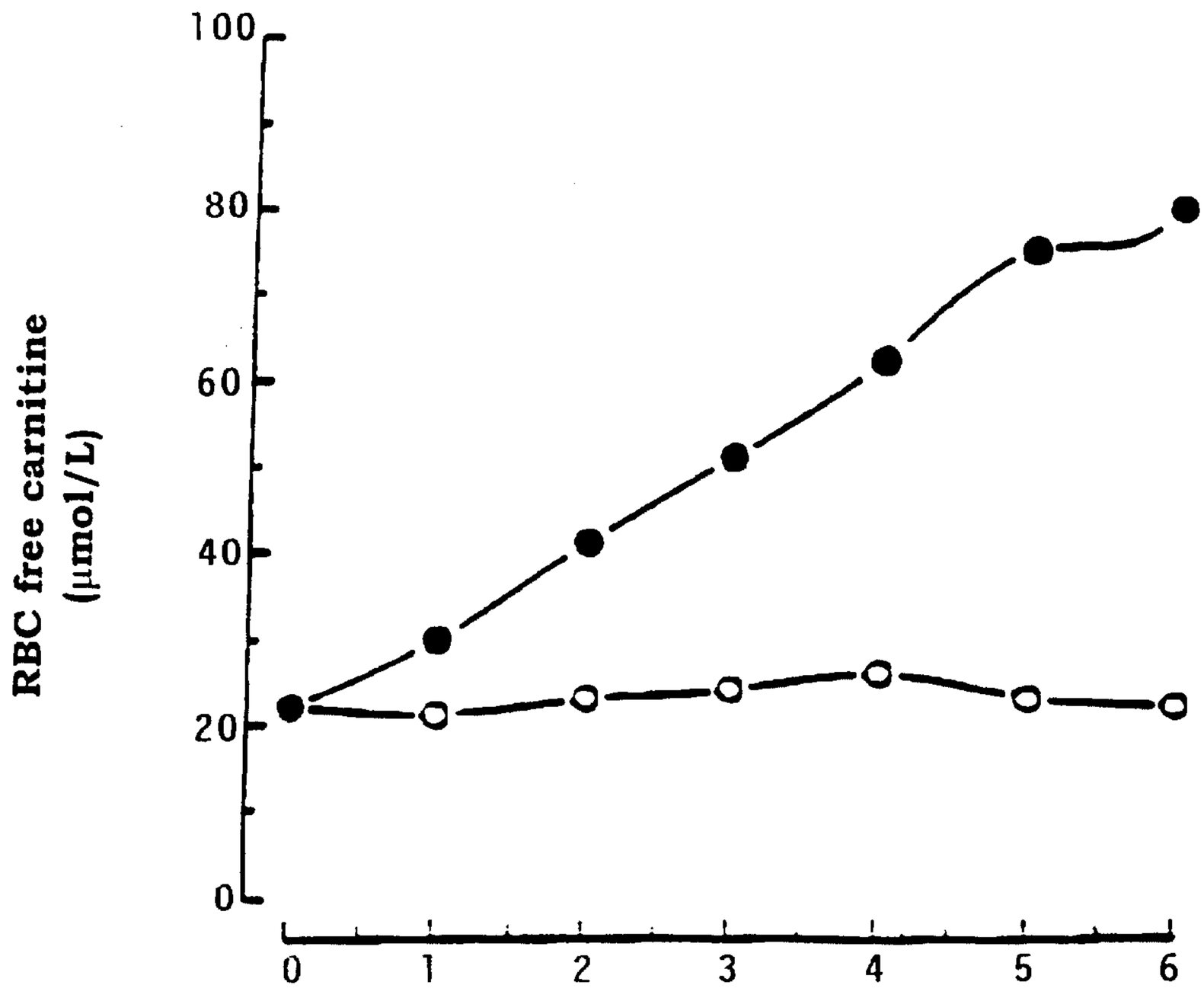
RBC free carnitine (μmol/L)
100
80
60
40
20
0
0
1
2
3
4
5
6
weeks of storage

Figure 2