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***Effects of ionizing radiation on
blood and blood components:
A survey***



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EFFECTS OF IONIZING RADIATION ON BLOOD AND BLOOD COMPONENTS:

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FOREWORD

Irradiation of blood and blood components is currently practised in developed and in a few developing countries. The main purpose of this process is the prevention of graft versus host disease in immunodeficient patients. This is achieved by the abrogation of T-lymphocytes, as a result of the specific effect of ionizing radiation at the low doses applied. As demonstrated by numerous studies, there are no accompanying significantly deleterious effects, and therefore the technique has acquired wide utilization in hospitals and transfusion services, in blood banks, and in related institutions. It is, however, not yet employed widely in developing countries.

Irradiation of blood and blood components is also possible at higher doses, provided the unavoidable deleterious effects are minimized or eliminated by protective measures such as, for instance, irradiation in the frozen state ("cryoirradiation"). Application of the treatment at the higher doses would achieve sterilization and inactivation of pathogenic micro-organisms in contaminated blood products. This would considerably increase the safety of transfusions, nowadays often associated with transmitted infectious diseases, as a result of contaminated blood products. Although routine screening of blood donations is now common practice, the possibility of transmission of infections is recognized owing to deficiencies and limitations of the existing screening methodology. This technique would, therefore, find wide application in the institutions mentioned above, as well as in the pharmaceutical industry processing or manufacturing blood products and blood product derivatives. It would be of particular value in developing countries in view of the existing procedures for testing of blood products for contamination such as, for example, from hepatitis and HIV.

The present publication reviews, in a comprehensive manner, the relevant literature on the effects of ionizing radiation on whole blood, blood cells, and other blood components. It presents the interested reader with sufficient information and data to facilitate rational decisions in relation to the feasibility of irradiation of blood and blood products for the purposes stated above. The IAEA expects that this can promote a wider use of the technology for improving health care practice in Member States, particularly in view of the recent spread of conventional as well as "modern" diseases which exert immunosuppressive effects in afflicted patients, with pathological consequences. Innumerable patients could thus benefit from this application of ionizing energy.

The IAEA appreciates the work performed by G.P. Jacobs (a consultant to international health care industries on the sterilization of pharmaceutical and biological products in general, and on their sterilization by ionizing radiation in particular) in compiling and evaluating the material comprised in this technical document, as well as that of a number of experts (see List of Contributors to Drafting and Review), whose valuable comments have been incorporated in this survey.

EDITORIAL NOTE

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1. INTRODUCTION

1.1. RATIONALE FOR BLOOD IRRADIATION

Currently the major application of blood and blood component irradiation is for the prevention of graft-versus-host disease in immunodeficient patients by the abrogation of T-lymphocytes. However, a potential application of this technology would be for the sterilization and inactivation of pathogenic microbes in contaminated blood products. Transfusion associated transmission of infectious diseases as a result of contaminated blood products is now well documented (for example, [1-4]) and routine screening of blood donations is now common practice. Nonetheless, it is recognized that the possibility of transmission of infections due to limitations of the screening methodology exists [5, 6].

The purpose of this report is to review relevant literature on the effect of ionizing radiation (essentially gamma and X-rays) on whole blood, blood cells and other blood components in order that a rational decision can be made on the feasibility of their irradiation whether for sterilization (or decontamination), or alternatively, for inactivation of a particular blood component such as, for example, lymphocytes in preventing graft versus host disease. It should be pointed out that while radiation doses for inactivation of T-lymphocytes, for instance, may be in the order of 10 to 50 Gy, doses used for sterilization purposes are generally a thousand-fold higher, i.e. in the 10 to 30 kGy range. Reviews have been published on the effects of sterilizing doses of ionizing radiation on polymers [7-13], pharmaceuticals [7, 14-28], cosmetic raw materials [29], and biological materials (other than blood components) [30-34]. However, no comprehensive review of the effects of ionizing radiation on blood products is readily available, although a bibliography was published some ten years ago by the Atomic Energy of Canada Ltd [35].

While many investigators have examined the *in vivo* effects of ionizing radiation or extracorporeal irradiation, this report is concerned essentially with the *in vitro* irradiation of blood and blood products.

For those readers of this report not too familiar with blood terminology, Table I presents a classification of blood components referred to in this report, together with the appropriate synonyms.

2. LEUKOCYTES

2.1. Granulocytes

In one of the earlier studies on the effects of radiation on leukocytes, Sokolov [36] demonstrated that X-ray doses of 90 Gy¹, caused the leukocyte count to drop by 30 percent when measured one day following irradiation.

In an electron microscope study of X-ray damage to frog blood cells, Lessler and Herrera [37] reported that following 10 Gy, there was increased vacuolization of the leukocyte cytoplasm.

¹Although many of the papers express radiation absorbed doses in rad, krad, Gy or kGy, all values have been converted here to the SI unit of gray (Gy), where 1 Gy (an absorbed dose of 100 J/kg) is equivalent to 100 rad.

TABLE I. CLASSIFICATION OF BLOOD CELLS AND COMPONENTS

Leukocytes (leucocytes; white blood cells)

Granulocytes

Neutrophils (or polymorphonuclear leukocytes)

Eosinophils

Basophils

Lymphocytes

Monocytes

Erythrocytes (red blood cells)

Platelets (thrombocytes)

Plasma

Plasma proteins

Albumin

Thrombin, etc.

Globulins

Alpha 1

Alpha 2

Beta

Gamma

Factor VIII (antihemophilic globulin)

Fibrinogen

Prothrombin

Plasma thromboplastin, etc.

While granulocytes are generally more radiation resistant than lymphocytes, they are less so than earlier studies had indicated, and there may therefore be some loss of granulocytic functional activity at doses recommended for prevention of post-transfusion graft-versus-host disease [38]. In fact, Valerius et al. [39] suggested that a 20 Gy radiation dose is likely to eliminate lymphocyte mitotic ability and prevent graft-versus-host disease, without significantly damaging granulocytes. On the other hand, Holley et al. [40] reported that granulocyte function in vitro was unaffected by radiation doses of up to 400 Gy. Stankova et al. [41], also studying granulocyte function, reported a slight decrease on oxygen consumption during phagocytosis following a 300 Gy radiation dose.

Whole blood irradiation at 50 Gy, followed by storage for 21 days, had no adverse effect on the granulocytic function [42]. In a later study, Button et al. [43] showed that granulocytes, irradiated at this dose level retained normal bacterial killing capacity, chemotactic mobility, and normal superoxide production after high dose stimulation. In contrast, another study showed that a 50 Gy radiation dose caused a 20 percent decrease in superoxide production by granulocytes [44].

Gamma irradiation of neutrophils at doses of up to 175 Gy produced no change in neutrophil aggregation, random migration or superoxide generation. Chemotactic response showed insignificant decline [45].

Holley et al. [40] reported little effect on neutrophil chemotaxis when cells were exposed in vitro to 50 Gy. As radiation dose increased to 500 Gy, chemotaxis dropped to 50 percent of the controls, and was minimal at 2500 Gy.

2.1.1. Effect on enzymatic function

Wolber et al. [46] and Wheeler et al. [46a] reported a normal oxidative response in neutrophils irradiated with up to 25 Gy.

In studies on enzyme systems in leukocytes, alkaline phosphatase activity is enhanced following a 500 Gy radiation dose [47]. The inherent aging process of leukocytes is probably enhanced by change in the cell envelope [47]. Alpha glycerophosphate dehydrogenase (one of the most important respiratory enzymes in leukocytes) activity is similarly enhanced by radiation. The glycolytic system in leukocytes remains intact at 500 Gy [47].

Maintenance of ATP content is of essential importance for normal functioning of cells. Cellular concentrates in saline were subjected to ionizing radiation in the range 0.5 to 100 Gy. At 10 Gy there was significant fall in ATP levels of granulocytes one hour after radiation exposure [48].

Buescher et al. [49, 49a] reported that a 50 Gy radiation dose altered oxidative metabolism of neutrophils and concluded that irradiation prior to transfusion contributed to defective oxidative metabolism, but that this effect is highly variable.

2.1.2. Chromosome aberrations

X-ray induced chromosome aberrations in human peripheral blood leukocytes were studied in vitro following different radiation doses [50]. There was no influence of harvest time or cell source on aberration rates. A significant increase in aberrations was seen only for doses of 0.3 and 0.5 Gy, partially supporting the theory that chromosome aberrations can be used as a practical biological dosimeter only for X-ray doses of 0.5 Gy or higher [50].

2.1.3. Effect on progenitor cells

In the case of whole body X-irradiation of granulocytic progenitor cells (CFU-C) of blood in dogs exposed to low doses, the number of CFU-C/ml of blood was significantly reduced within one day to 15-43 % of that in normal blood after a dose of 0.44 Gy, and 1-6% of that in normal blood after 0.88 Gy. Following a 0.22 Gy radiation dose, there was no reduction in the number of cells [51].

2.1.4. Effects of granulocyte storage

Granulocyte functional capacities were not affected following gamma-irradiation at 15 Gy and storage for up to 24 h at 4°C. There was moderate loss of activity after 48 h storage. Chemotaxis appeared to be the most sensitive detector of cellular damage of stored granulocytes [52].

2.2. LYMPHOCYTES

Lymphocytes have been found to be one of the most radiosensitive mammalian cells [53]. Although irradiation has been shown to alter neither lymphocyte count nor their viability

[54], Wong et al. [54] reported impairment of their function following a 30 Gy radiation dose, as evidenced by a decrease in response to phytohaemagglutinin and mixed leukocyte culture. In fact, Pelszynski et al. [55] reported that to effectively inactivate lymphocytes in red blood cell units, a minimal gamma radiation dose of 25 Gy is required.

In a long term study using light and electron microscopy, discontinuous extracorporeal blood gamma-irradiation was undertaken. Following a particularly high dose of 4665 Gy, lymphocytes in peripheral blood decreased from 6900/ μ l to 500/ μ l, revealing a complete dissolution of the nuclei. Histological examination revealed severe atrophy of the whole lymphatic tissue [56].

As with other biological systems, anoxia protects lymphocytes from radiation injury [57-59]. Its manifestation on chromosomal aberrations is discussed below. Biological factors, such as the stage of the cell cycle at the time of irradiation or the relationship between irradiation and antigen challenge, could also play an important role in determining the ultimate effect [38].

The time interval between irradiation and transfusion of lymphocytes would appear to be critical. For example, Button et al. [42] reported that irradiation of whole blood, followed by storage for 21 days, resulted in more than a 95 percent reduction in lymphocytic response following a 50 Gy radiation dose, and 100 percent inactivation after 75 Gy.

As described earlier for granulocytes [48], concentrates of lymphocytes in saline were subjected to ionizing radiation at doses of up to 100 Gy. The lowest radiation dose causing significant fall in ATP levels one hour after radiation exposure was 5 Gy [48].

2.2.1. Effect on T-lymphocytes and B-lymphocytes

As summarized by Lavin and Kidson [60], B-lymphocytes are more radiosensitive than T-lymphocytes, although it is well recognized that resistance to ionizing radiation varies in the subpopulations of both cell types, with mitogen stimulated T-cells being more resistant to radiation than quiescent cells.

It has been postulated by Conard [61] that the hypermetabolic state of activated T-cells might protect them from interphase death, but not from mitotic death. This increased protection has been attributed by Lavin and Kidson [60] to the induction of DNA repair enzymes in the stimulated lymphocytes.

In a study on the effect of in vitro X-ray irradiation (10 Gy) on human peripheral blood T-lymphocytes, and the influence of their suppressor activity on the concanavalin A (Con A) induced suppressor system, the suppressor activity gradually increased with lapse of time from irradiation to the suppressor cell assay. Suppressor T-lymphocytes were resistant to X-ray irradiation and independent of DNA synthesis. On the other hand, irradiation-induced enhancement was minimal in cultures incubated with Con A, regardless of the irradiation time [62].

2.2.2. Chromosomal changes in irradiated lymphocytes

Numerous studies have been carried out on the effects of ionizing radiation on chromosome changes in blood components. From X-irradiation (0.25 to 5 Gy) of human

peripheral blood lymphocytes, Todorov [63] found a non-linear relationship between radiation dose and chromosome aberrations of the dicentric, interstitial deletion and ring type, and a linear relationship between radiation dose and chromosome aberrations of the gap and break type. The frequency of cell changes increased as a linear function of the dose, while the frequency of breaks increased as a non-linear function of the dose.

In another investigation of a relationship between yields of micronuclei and radiation dose, micronuclei were induced in lymphocytes by exposing human blood samples in vitro to various doses of Cs-137 gamma-rays. It seems, according to Balasem and Ali [64], that there is a correlation between the yields of micronuclei in mononuclear cells and the corresponding doses of radiation.

Similarly, experiments were conducted by Kligerman et al. [65] on the chromosome damaging effects of Co-60 gamma radiation on human peripheral blood lymphocytes (PBLs). Either whole blood or isolated and pelleted mononuclear leukocytes (MNLs) were irradiated in a Co-60 unit to yield exposures of 1, 2, 3, or 4 Gy. The data best fitted to a quadratic function. Human PBLs did not show a marked difference whether irradiated in whole blood or as MNLs in tissue culture medium.

Erexson et al. [66] analyzed micronuclei in cytochalasin B-induced binucleated (BN) cells in human peripheral blood lymphocytes (PBLs) X-irradiated as whole blood at doses of 0.38, 0.75, 1.5 or 3 Gy. All cultures were harvested after 52 hours post-irradiation and micronuclei counted. Data were fitted to a linear/quadratic model. Significant dose-dependent increases in the percentage of micronucleated cells and the number of micronuclei per BN cell were observed. The correlation between the percentage of cells with micronuclei and those with chromosome aberrations was high (r^2 greater than 0.95).

An excess in the frequencies of reciprocal translocations relative to those of dicentrics was induced in human blood lymphocytes by X-irradiation (0.5, 1.0 and 2.0 Gy), as measured using the fluorescence in situ hybridization (FISH) technique, at the first cell division after irradiation (translocation: dicentric approximately 60:40). However, when the same metaphases were also evaluated sequentially by a conventional staining method, the ratio of about 50:50 was restored. This was due in part to misclassification of certain dicentrics as reciprocal translocations by the FISH technique [67].

In another study [68], the low dose response for X-ray induced chromosome aberrations in lymphocytes irradiated with radiation doses ranging from 0 to 300 mGy was investigated. No significant decrease was noted in the aberration yield below the level for zero dose controls for the irradiated cells. This was in contrast to other reported results showing a dip below control levels for aberrations around 4 mGy, which was considered to represent low dose stimulation of enzymic repair. A significant increase in the aberration yields was noted at doses of 20 mGy and above. The authors concluded that it is not certain, due to uncertainties in the data, whether a very low dose plateau exists. If so, it extends only up to the 10 mGy point. The data fit well over the 0 to 50 mGy range into a linear model. The probabilities of fit to the quadratic model over the range 0 to 300 mGy were considered reasonable, but there were large errors in the beta terms. The authors concluded that at doses up to 10 mGy, a plateau level may exist, and at doses above 10 mGy the aberration yields are elevated. An unexpected finding was that some lymphocytes contained more than one exchange aberration, which may be an indication that a small subset of cells were particularly susceptible to the induction of aberrations by low doses of irradiation [68].

Two methods of using frequencies of micronuclei as quantitative indicators of X-ray induced chromosomal aberrations were compared in human peripheral blood lymphocytes which had undergone one cell division [69]. The first method involved incorporating bromodeoxyuridine (BrdU) in cultured lymphocytes, with differential staining used to detect those cells which had undergone one cycle of DNA synthesis. The second method involved the inhibition of cytokinesis by cytochalasin-B and resulted in the production of binucleated cells. Micronuclei were scored in stained preparations, and chromosomal aberrations were classified as acentric fragments or exchanges (dicentric, trisentric, and centric rings). The cytochalasin block method was found to be more efficient with a capacity to detect between 60 and 90% of the induced fragments. The yield of micronuclei reflected those classes of acentric fragments both associated with and independent of exchange type aberrations. The authors conclude that the frequency of micronuclei detected by the cytochalasin block method is suitable as an indicator of the frequency of chromosomal aberrations induced by ionizing radiation [69].

Other studies on radiation induced micronuclei in lymphocytes have been undertaken by, *inter alia*, Huber and his colleagues [70-72], Vral et al. [73], Erexson et al. [74], Aghamohammadi and Henderson [75], Nugis and Pyatkin [76], Pleskach et al. [77], Das and Sharma [78], Sanford et al. [79], and Shchedrina et al. [80].

Golub et al. [81] investigated the effect of inhibitors of DNA and protein synthesis on the chromosome aberration yield in a human lymphocyte culture exposed to gamma and neutron irradiation at different stages of the mitotic cycle.

A comparative study of survival of peripheral blood lymphocytes from neonatal umbilical cord blood or from children and adults after exposure to gamma irradiation (up to 4 Gy) was made [82]. Mean inactivation doses of radiation were calculated from cell survival rates in the cloning assay. Cord blood samples showed a strong tendency toward lower mean inactivation dose (1.54 Gy) relative to comparisons (1.90 Gy). Based on survival rates at different doses, it was noted that cord blood samples showed increasing radiosensitivity above 1 Gy. The authors conclude that these results confirm greater T-lymphocyte radiosensitivity in newborns, which may have implications for prenatal radiation protection [82].

The induction of DNA damage following gamma ray exposure at doses of 0.05, 0.10, 0.25, or 0.50 Gy, was investigated in isolated lymphocytes and granulocytes as well as in unfractionated leukocytes in whole blood. DNA single strand breaks and alkali labile sites were determined using the alkaline single cell gel electrophoresis technique. Linear and dose dependent increases in DNA damage were seen in all three cell preparations compared with controls. No significant differences in the dose response was seen between isolated lymphocytes and granulocytes; however, these cell populations had significantly more damage than that seen in whole blood leukocytes. The distribution of damage among the cells was found to be essentially independent of radiation dose and cell population. The authors conclude that the irradiation of leukocytes in whole blood provides protection, at least in part, against radical-induced DNA damage [83].

2.2.2.1. *Oxygen effect and chromosomal aberrations*

In common with other biological systems, Watson and Gillies [59] showed that dose-effect curves for lymphocytes were somewhat different for cells X-irradiated in the presence and absence of oxygen. The two conditions of irradiation also affected the dependence of chromosomal aberration yield on the time at which cells were fixed. For cells

irradiated in oxygen, the yield of "unstable" aberrations was the same, whether they were incubated for 50 or 60 hours, whereas cells irradiated in anoxia manifested the maximum number of aberrations only after the longer time interval.

2.2.2.2. Effect of storage on chromosomal abnormalities

Sharma and Das [84] showed that in heparinized whole blood stored for 72 h at 5°C, or 48 h at 22°C, or 24 h at 37°C, then X-irradiated at either 2 or 3 Gy, the yield of dicentrics and other chromosomal aberrations in lymphocytes scored in the first post-irradiation metaphase did not vary significantly.

The yield of chromosomal abnormalities was studied in cultured lymphocytes irradiated in vitro after different periods of storage in plastic or glass containers. Blood stored for 48 and 72 hours displayed a higher yield of abnormalities after irradiation than that stored for 24 hours. No differences could be discerned in this pattern between blood stored in plastic containers and that stored in glass containers [85].

2.2.3. Graft-versus-host disease

The prevention of graft-versus-host disease is probably one of the major reasons (if not the major reason) for blood irradiation today. Graft-versus-host disease is a rare complication of transfusion that results from the engraftment of the residual T-lymphocytes present in cellular blood components. Clinically, it is characterized by fever, liver function abnormalities, profuse watery diarrhoea, and an erythematous skin rash which may progress to generalized erythroderma and desquamation. Nearly ninety percent of patients with post-transfusion graft-versus-host disease will die of acute complications of the disease [61, 86-88a]. It has been recognized that immunodeficient patients are at risk from the disease.

Gamma irradiation is the procedure routinely used to prevent transfusion associated graft-versus-host disease due to abrogation of in vitro proliferation of T-lymphocytes. This procedure was first described in animal studies in 1959 [88b] and 1960 [88c], and in humans a year later [88d]. Furthermore, according to Leitman and Holland [86], prophylactic irradiation of blood products prior to transfusion is presently the most efficient way to prevent post-transfusion graft-versus-host disease.

2.2.3.1. Controversy over the appropriate radiation dose

In 1974, Sprent et al. [89] reported that as little as 5 Gy abolishes mixed lymphocyte culture reactivity, prompting them to suggest that this radiation dose should be sufficient to prevent transfusion associated graft-versus-host disease. On the other hand this level of radiation has little effect on the response to mitogens.

At the same time, Parkman et al. [90] recommended that blood components be irradiated prior to transfusion into infants previously given intrauterine transfusions.

There appears to be some controversy on the radiation dose required to suppress allogenic lymphocyte proliferation. Many groups of investigators, including Thomas et al. [91], De Dobbeleer et al. [92], Lowenthal et al. [93], Graw et al. [94], Leitman and Holland [86], and more recently Holland [95], have reported and, in many cases, recommended the use of a 15 Gy radiation dose as an adequate dose to prevent transfusion associated graft-versus-host disease. On the other hand, Mc Cullough et al. [96] recommended a 60 Gy dose.

Fagiola et al. [97], who found that following irradiation of whole blood, a 8 Gy dose inhibited ³H thymidine uptake by 95 percent of lymphocytes, are of the opinion that 16 Gy is sufficient to prevent graft-versus-host disease [97].

Van Bekkum [98] suggests that in cases where oxygenation of blood cannot be controlled, the radiation dose be doubled to 30 Gy. Similarly, Coifman and Meuwissen [99] suggest using a 30 Gy dose to reduce the number of viable immunocompetent lymphocytes by a factor of 10¹³, in cases where the number of lymphocytes reaches 10¹¹.

Weiden et al. [100] state that irradiation of blood products can be performed with "minimal inconvenience, time and relatively modest expense". They chose 15 Gy based on earlier studies. This dose was adequate to markedly inhibit proliferation of lymphocytes without impairment of red cell, platelet or granulocyte function. However, they cite other studies supporting radiation doses higher than 15 Gy [100].

In a much quoted study on the functional properties of formed elements of whole blood following radiation doses of 5 to 200 Gy, Button et al. [43] demonstrated that irradiated lymphocytes retained only 1.5 percent of their ³H thymidine uptake after 50 Gy, and none after 75 Gy. Red blood cells, stored for 21 days and then irradiated at 50 Gy, had the same survival as unirradiated controls. In contrast, 50 Gy reduced platelet yields. However, transfused irradiated platelets produced the expected increases in platelet counts and controlled haemostasis in thrombocytopenic patients. Granulocytes, following 50 Gy, retained normal bacterial killing capacity, chemotactic mobility, and normal superoxide production after high dose stimulation. In summary, the functional qualities of cellular blood components other than lymphocytes were not compromised at 50 Gy. The authors thus concluded that irradiation may be an effective means of controlling incidence of graft-versus-host disease in immunosuppressed patients that are to receive bone marrow transplants. Their practice is therefore to irradiate all blood components for use in such patients, beginning five days prior to transplantation, and, following the engraftment procedure, their practice is to irradiate with a 50 Gy dose.

In another study in support of irradiation at 50 Gy, Button [101] showed greater inhibition of PHA-stimulated blastogenesis of peripheral blood lymphocytes by 50 Gy than by 15 Gy, without detrimental effects on stored red blood cells or platelets.

Following reports of graft-versus-host disease occurring in patients treated with chemotherapeutic agents after the transfusion of a single unit of blood, consideration was given to irradiation of all blood components [102-104]. Lowenthal et al. [106], in view of the ongoing controversy concerning the possible anti-leukaemic effects of granulocyte transfusions, and until the relative importance of the benefits and of the deleterious effects of cells with the potential for engraftment is determined, recommend that all cellular blood products intended for administration to acute leukaemia patients undergoing intensive cytoreductive chemotherapy be irradiated. Following a subsequent clinical outcome, this group suggests that irradiation at 15 Gy is insufficient to prevent transfusion associated graft-versus-host disease, and that 30 Gy may be more appropriate [106] (the Austrian guidelines, for instance, recommend this dose [106a]).

On the other hand, the Standards Committee of the American Association of Blood Banks [107, 107a] has recommended a dose of 15 Gy.

As stated by Pisciotto [38], the current controversy is not over whether irradiation should be undertaken, but rather over the radiation dose that is sufficient to completely

eliminate the hazard of graft-versus-host disease and still preserve the functional capacity of the blood components, and over the types of recipients of irradiated products. On the other hand, Van Bekkum [98] had recommended that irradiation be restricted to platelet transfusions with a lymphocyte contamination greater than a critical level of 0.5 to 1×10^8 / kg body weight [98].

In a review on graft-versus-host disease and methods of inhibiting lymphocyte proliferation, including by irradiation, Brubaker [108] concludes that doses of between 15 and 75 Gy appear adequate in preventing graft-versus-host disease, with an optimum dose of 50 Gy. He states that helper T-lymphocytes are not sensitive to 30 Gy, whereas suppressor and cytotoxic T-lymphocytes are sensitive to this radiation dose. Following exposure to 15 Gy, the blastogenic response to tetanus toxoid and allogenic lymphocytes (antigens used to assess human cellular immunity *in vitro*) is reduced by 95 percent, and following 35 Gy it is completely ablated. Irradiation inhibits T-lymphocytes, but has no effect on erythrocyte and granulocyte function or survival. He recommends an optimal radiation dose of 35 to 50 Gy.

According to Anderson et al. [109, 109a], the only current process that is effective in preventing transfusion associated graft-versus-host disease, is gamma irradiation of the blood products. A dose as small as 5 Gy can abrogate the response of lymphocytes to allogenic cells in a mixed lymphocyte culture, and 15 Gy can reduce the response to mitogen-induced stimulation by 90 percent. They recommend that the lowest dose capable of inhibiting lymphocyte proliferation (15 to 25 Gy) be used to irradiate blood components before transfusions. A survey which they undertook showed that 97 percent of the institutions use radiation doses of between 15 and 35 Gy. Leitman [110] is also of the opinion that gamma irradiation is the only proven method for prevention of transfusion associated graft-versus-host disease. In an editorial, she states that a dose of 25 Gy is sufficient to prevent graft-versus-host disease [111].

In a related study, Valerius et al. [39] reported a study on the effect of *in vitro* gamma-irradiation from a Cs-137 source (despite the title!) on elements of lymphocyte and granulocyte function, to determine a radiation dose to block lymphocyte function without affecting the granulocytes. Lymphocyte blast transformation, after stimulation with mitogens (microbial antigens), was reduced by 90 percent after a 15 Gy dose, and by 97 percent after 50 Gy. Coifman and his colleagues [112], however, note that mitogen responsiveness, which only detects the ability of cells to recognize and react to stimulus, may not be a valid measure of the immunocompetence of irradiated cells. According to Valerius et al. [39], mobility was the function of polymorphonuclear leukocytes most affected by irradiation, being slightly but significantly reduced after irradiation with 100 to 200 Gy. The bactericidal activity was only reduced after a dose greater than 400 Gy, while the hexose monophosphate shunt activity and the myeloperoxidase activity was largely unaffected with doses of up to 1200 Gy. The authors [39] conclude that irradiation of leukocytes with a dose of 20 Gy is likely to prevent graft-versus-host disease without causing any apparent damage to the polymorphonuclear leukocytes.

There is no doubt that dissimilar irradiation conditions could well have contributed to the somewhat conflicting findings of the various studies. As summarized by Luban and Ness [113], factors that may influence radiation response include differences in lymphocyte subpopulations [87], sensitivity to cytolysis [88], oxygen tension during irradiation [114], volume irradiated, container configuration, and influence of plastic container material [113].

2.2.3.2. Irradiation of blood components for neonates

With regard to the irradiation of blood components for transfusion to neonates, it is questionable whether gamma irradiation to prevent graft-versus-host disease is needed for such patients, other than for high risk groups for congenital immunodeficiency diseases. In view of the rarity of transfusion associated graft-versus-host disease during infancy, it is the opinion of Strauss and his colleagues [115] that it is not appropriate to irradiate all blood components intended for neonates. In fact, as discussed later, it would appear that 41 percent of the institutions giving neonatal transfusions do not irradiate any of their blood products [115-116].

2.2.3.3. Potential for malignant transformation

One particular theoretical concern raised by Pisciotto [39], is the potential for malignant transformation of irradiated lymphocytes which may survive in the recipient. Although there have been no experimental studies conducted to address this issue, it may be an important consideration when deciding whether irradiated blood can be transfused into a patient not at high risk.

This dilemma has previously been considered by Leitman and Holland [86] who argue that since the mean lethal dose of radiation for lymphocytes and haematopoietic stem cells is less than 2 Gy [117], well below the dose used in irradiating blood products, there is no possibility for sustained proliferation of cells with radiation-induced carcinogenic potential. They conclude that since all such cells will die during subsequent mitosis, and thus, if an irradiated unit is no longer required for its intended recipient, it may be safely transfused to another patient not necessarily requiring an irradiated product [86], a conclusion, incidentally, not considered by Luban and Ness [113] to be "appropriately cautious".

Related and other concerns have also been raised by Luban and Ness [113]. They point out that while they were not aware of adverse reactions from irradiated products, their theoretical causes for concern include the possibility that radiation sensitive pluripotential stem cells in stored and peripheral blood may self replicate and differentiate into different committed cell lines in the host, or that irradiation could alter DNA structure or repair processes [118], or could produce sister chromatid exchanges [119]. Luban and Ness [113] suggest that such exchanges might predispose to primary or secondary malignancies and to chromosomal damage, and that this potential problem would be of greatest concern when irradiated blood was used for neonates with normal expected life spans. However, they conclude that despite their theoretical concerns, they have no documented evidence that irradiated blood products are of any danger to a recipient not requiring irradiated blood.

2.2.4. Adaptive response

Olivieri et al. [120] first reported that pre-exposure of human blood lymphocytes to low doses of radioactive thymidine led to significant reduction in chromosome damage induced by subsequent challenge with a higher dose of X-rays. In a subsequent study, human peripheral blood lymphocytes exposed to a single adaptive dose of 0.01 Gy X-rays, or two adaptive doses of 0.01 Gy each, were found to be equally resistant to induction of chromosome damage by subsequent challenge with a high dose of 1 Gy, in comparison to blood lymphocytes not exposed to the adaptive doses(s). There was a significantly reduced incidence of chromatid and isochromatid breaks. Results indicated the presence of an inducible chromosomal repair mechanism in human blood lymphocytes, and confirmed the

observations made by earlier investigators [121]. In a similar study, exposure of human blood lymphocytes to an X-ray dose of 0.005 Gy significantly reduced cytogenetic damage induced by subsequent challenge with a 1.5 Gy dose [122].

Factors affecting the role of adaptive X-radiation doses on effects of subsequent larger challenge doses to human lymphocytes include the size of the adaptive dose, the time interval between the adaptive and challenge doses, dose rate and the quality of radiation [123-124]. It has been shown by several groups of investigators [123, 125-126] that the adaptive response of human lymphocytes to X-rays can be negated by 3-aminobenzamide, an inhibitor of poly (ADP-ribose) polymerase, if added to lymphocyte cultures immediately following the challenge dose, thus implicating a possible role for repair enzyme(s) in the process.

2.2.5. Radiation repair

Human peripheral blood lymphocytes are one of the most radiosensitive mammalian cells. Michel and Laval [127] tested whether this radiosensitivity was associated with the persistence of unrepaired DNA damage in gamma-irradiated lymphocytes. Results show a limited repair capacity in resting human lymphocytes after gamma irradiation.

The effect of X-irradiation on growth of T-lymphocyte colony forming units (CFU) from human peripheral blood was investigated [128]. It was not the number of activated T-lymphocyte CFU, but the number of cell cycles of colony forming cells that was reduced by X-irradiation. Apparently, T-lymphocyte CFU belong to a relatively radio-resistant cell population within the PHA (phytohaemagglutinin)-responsive lymphocytes. Kinetic studies revealed that colony growth following irradiation was delayed mainly during the phase of the first cell cycle. Preculture of the cells for 24 h after irradiation with 12 Gy in the absence of PHA, caused total inhibition of colony growth in the subsequent agar culture. In the presence of PHA no inhibition was observed. This finding indicates a repair mechanism from radiation damage of lymphocytes stimulated by PHA [128]. For further reports on repair of radiation-induced damage in lymphocytes the reader should refer to the work of Boerrigter and Vijg [129], and Sinover [130].

2.3. MONOCYTES

A 25 to 50 Gy dose significantly decreased the survival and growth of human monocytes in culture. The irradiated monocytes killed *Listeria monocytogenes* at a slower rate than the controls [131].

3. ERYTHROCYTES

Mature non-nucleated erythrocytes, in comparison to other blood components, would appear to be relatively resistant to radiation damage [for example, 42, 86].

3.1. RADIATION EFFECT ON ERYTHROCYTE MORPHOLOGY

X-irradiation of erythrocytes at 200 Gy failed to alter their morphology, osmotic and mechanical fragility, or glycolytic activity. However, incubation at 37°C for 24 h slightly increased their fragility [132].

In an electron microscope study of X-ray damage to frog blood cells, Lessler and Herrera [37] reported that following 10 Gy, erythrocytes became swollen, nuclei were bulging,

and there was disruption of the internal structure of the mitochondria. In another study, Lessler [133] showed that a lower level of X-rays (1 Gy) was sufficient to cause cytological abnormalities to bullfrog erythrocytes.

3.2. HAEMOLYSIS AND ERYTHROCYTE MEMBRANE DAMAGE

One of the first reports of radiation-induced haemolysis was in 1904 when Henri and Mayer [134] reported that blood could be haemolysed by irradiation with radium. The same effect was later shown with X-rays [135]. A 20 kGy dose of gamma irradiation was reported to cause disruption of the polypeptide protein chain of haemoglobin in erythrocytes [136].

Ambe et al. [137] reported that 1 kGy irradiation caused a 2 percent loss in cattle haemoglobin in anoxic aqueous solution, when expressed as total nitrogen in scission products. A 10 kGy radiation dose caused a 15 percent loss, and 600 kGy caused a 29 percent loss. At 100 kGy there was complete destruction of the chromophore group and the functional properties of the haemoglobin, with a 30 to 45 percent loss of these properties at 10 kGy. The essential effects of radiation in aqueous solution were formation of insoluble protein aggregates, and formation of scission products.

While there was no decrease in the number of red blood cells following doses of X-rays of up to 97 Gy, haemolysis was evident at doses greater than 60 Gy [36].

In X-irradiated suspensions of erythrocytes at concentrations of 0.5 to 5 percent, haemolysis was independent of the suspending medium. However, at cell concentrations of less than 0.5 percent, haemolysis was dependent on the type of suspending medium [138].

In a further study [139], haemolysis was evident following the gamma irradiation, in the dose range 160 to 500 Gy, of erythrocytes suspended in isotonic sodium chloride solution. No haemolysis was observed in isotonic choline chloride solution (although there was a loss of potassium ions), nor in hypertonic sodium chloride or potassium chloride. The results indicate that radiation induced haemolysis is an osmotic effect due to destruction of the barrier to sodium and potassium ions (caused by a decrease in membrane sulphhydryl groups), and movement across the cell membrane [139].

Potassium and haemoglobin levels in X-irradiated rat erythrocytes were greatly reduced at 2 kGy followed by incubation for 20 h at 4°C or 37°C [140].

Examining the nature of the membrane injury in gamma irradiated erythrocytes in the dose range 2 to 200 Gy, Shapiro and Kollmann [141] concluded that the sulphhydryl group is the major target in radiation-induced alteration of sodium and potassium ion permeability. Using fluorescent probes, Yonei and Kato [142] found that X-irradiation readily induces significant changes of the erythrocyte membrane structure.

X-irradiation of intact red blood cells up to 450 Gy caused aggregation of membrane proteins, due in part to disulfide bridges, which increased with post-irradiation incubation. The effect was marked at doses above 110 Gy. Irradiation at 450 Gy followed by incubation for 4 h at 37°C, resulted in 26 percent of the cells becoming echinocytes (crenated shape erythrocytes), compared to 6 percent echinocytes in the unirradiated control [143].

Washed erythrocytes, X-irradiated at 2.4 kGy, were examined by Zacek and Rosenberg [144] for changes in their membranes. There were considerable destructive changes including destruction of lipoproteid, the basic structural unit of the membrane.

In one of the earlier studies on cell membrane permeability of erythrocytes, Ting and Zircle [145-146] reported on the kinetics of the diffusion of salts into and out of X-irradiated erythrocytes. The X-irradiation of erythrocytes was reported by this group to cause osmotic disturbances. They showed that at 330 Gy, the effect of X-rays was not due to haemolytic disturbances in the red blood cells but rather to a "disturbance within the cells". This manifested itself in slow swelling of the cells [145-146]. Another early study was that of Solomon [147], who investigated the permeability of red cells to water and ions.

An *in vitro* study on the effect of X-rays on movement of sodium in human erythrocytes, showed a loss of sodium/potassium ion balance with entry of sodium ions into the erythrocytes, and exit of potassium ions, following radiation doses in the range of 8.9 to 89 Gy. This phenomenon was due in part to discontinuation of membrane integrity [148]. Radiation-induced changes in the properties of cell membranes, resulting in loss of ability to regulate electrolyte balance and changes in permeability, have been reported for red blood cells by Coggle [149].

Irradiation of erythrocytes with a 20 Gy dose caused a significant increase in the external potassium ion concentration and internal sodium ion concentration. The change was attributed to increased permeability of the red blood cell membrane lipid bilayer to sodium and potassium ions. These changes could be reversed by incubating the cells at 37°C [150].

Whole body X-irradiation of rabbits with a dose of 6 Gy produced a rise in the concentrations of calcium and magnesium ions in whole blood and in erythrocytes, due to disturbances in permeability of the membrane to metal ions and other cellular components [151].

Erythrocytes, X-irradiated at 430 Gy and then stored for 26 h at either 24 or 38°C, showed no significant haemolysis. However there was leakage of potassium ions out of the cells and reciprocal penetration of sodium ions. The rate of loss of potassium is roughly proportional to the dose. For example, at 200 Gy at 24°C there was a 0.4 percent loss per h of the initial cell potassium concentration. This loss approximately doubled for every 200 Gy increase in radiation dose. The presence or absence of oxygen did not affect potassium loss [152-153].

A study of the protective effect of various concentrations and molecular weights of polyvinylpyrrolidone (PVP) on haemolysis of sheep erythrocytes irradiated up to 13 kGy, and stored for 1 month at 4°C, was reported by Eisenberg et al. [153a]. A 10 to 15% concentration of PVP reduced haemolysis from 90 to 10%.

3.2.1. Necessity for washing transfused erythrocytes

For reduction of potassium plasma levels in stored irradiated units, Ferguson [154] does not consider it necessary for irradiated red blood cells to be routinely washed and reconstituted with fresh frozen plasma. He does not consider a potassium ion concentration, which he estimates at 0.09 mmol (0.09 mEq), to warrant such treatment. Similarly, Avoy [155], does not see the necessity for washing irradiated red blood cells, and reconstituting with fresh frozen plasma.

On the other hand, Rivet et al. [156], in a study on potassium levels in irradiated blood, reported values of 26.2 mmol/l (26.2 mEq/l) potassium ion concentration for red cell concentrates irradiated at 20 Gy, then stored for 4 days, compared to a value of 12.6 mmol/l

for the control samples. However, when the procedure was reversed and irradiation followed 4 days' storage, values were 17.0 mmol/l for the irradiated samples, and 11.4 mmol/l for the controls. They recommended manual washing of the red cell concentrate and reconstitution with fresh frozen plasma to reduce the potassium levels.

In an editorial comment on the issue of routinely washing irradiated red blood cells before transfusion, Strauss [157] considers the procedure unwarranted.

3.3. METABOLIC EFFECTS

Bresciani et al. [158] showed that the ATP, ADP, and AMP content of erythrocytes 1 h after X-irradiation with 8.9 or 17.8 Gy was not changed. However, higher doses of 44.5 or 89 Gy caused a decrease in ADP and AMP content, but increased the ATP content of the erythrocytes. They suggest that X-irradiation produces its effect by interfering with membrane metabolism. A study by Kotelba-Witkowska et al. [48] showed that irradiation of erythrocyte concentrates in saline in the dose range 0.5 to 100 Gy resulted in the ATP remaining "nearly unchanged".

Carbonic anhydrase activity of erythrocytes remained unchanged following 2 kGy X-rays, although their haemolytic resistance diminished [159].

Sheppard and co-workers [152-153] reported that at 500 Gy there was no significant decrease in the rate of sugar utilization or lactate production. They further reported that at 420 Gy there was no significant difference in the rate of hydrolysis of added acetylcholine [152-153].

3.4. OTHER EFFECTS

Pelszynski et al. [160] used limiting dilution analysis to measure clonable T-cells, following 5 to 30 Gy of gamma irradiation delivered in situ to ADSOL-preserved red blood cell (RBC) units in blood bags. In a series of experiments using red blood cell units irradiated within 24 hours after collection, 15 Gy inactivated more than $4 \log_{10}$ of T-cells; however, viable T-cells were detected in all experiments. With 20 Gy, more than a $4.7 \log_{10}$ decrement in T-cell growth occurred in 7 out of 8 experiments. With 25 or 30 Gy, no T-cell growth (more than $5 \log_{10}$ depletion) was detected. Comparable effects were observed in the standard PL 146 plastic container and in the recently developed PL 2209 plastic container. T-cell inactivation, as a function of gamma irradiation dose, was similar when either a Cs-137 or a linear accelerator source was used. T-cells isolated from ADSOL-preserved red blood cell units after storage for 7 and 21 days, although reduced in number as compared with a fresh unit stored for 24 hours, were viable, capable of proliferation, and susceptible to inactivation by gamma irradiation. Using a sensitive in vitro assay for T-cell proliferation, they found that a gamma irradiation dose of 25 Gy may be required to completely inactivate T-cells in red blood cell units.

Morales-Ramirez et al. [161] have investigated the induction of micronuclei by acute and chronic exposure to gamma rays in murine polychromatic erythrocytes in vivo. Radiation induced micronuclei in mouse erythrocytes at very low dose rates have been examined by Zatterberg and Grawe [162].

3.5. EFFECTS OF STORAGE ON IRRADIATED CELLS

A number of investigators have determined the effect of storage on irradiated erythrocytes. Unfortunately, it is not a simple task to make a comparison between their findings due to the different experimental conditions, including criteria used in assessing any change, radiation dose, storage temperature and duration, and whether irradiation was carried out before or after the storage period.

3.5.1. Post-irradiation storage

Hilger et al. [163] determined the effect of storage on irradiated (35 Gy) red cell units. The results of their findings, in comparison to stored unirradiated controls, were as follows: ATP decreased from 402 (unirradiated) to 332 $\mu\text{M/l}$ (irradiated) by day 42 of storage, a change considered minimal; potassium increased from 40 to 57 mmol/l by day 42 - the increase by day 14 was from 17 to 37 mmol/l, a change of 1.7 to 2 mmol per unit was considered clinically insignificant; sodium decreased from 139 to 131 mmol/l by day 42, a minimal decrease; pH dropped from 6.52 to 6.48 by day 42, a minimal drop; lactate dehydrogenase (LDH) increased from 75 to 91 U/l by day 42, a minimal increase; and plasma free haemoglobin (PFH) increased from 400 to 520 mg/l by day 42, a minimal increase. They conclude that storage for 28 days is acceptable [163].

In a study on the effect of a 30 Gy gamma radiation dose within 4 hours of blood collection, followed by storage at 4°C for 42 days, Davey et al. [164] reported that haemoglobin increased by some fifty percent in the irradiated unit; potassium ion concentration increased from 43 to 78 mEq/l; ATP decreased from 2.1 to 1.9 $\mu\text{M/g Hb}$; 24 hour post-transfusion red cell recovery dropped from 78.4 to 68.5 percent (75 percent is an acceptable level recovery). Because of the damage to the red cells, they concluded that following a 30 Gy dose, blood may not be stored for 42 days [164].

Gamma irradiation at 15 Gy, followed by storage at 4°C for 6 days, then frozen and stored at -75°C for 56 days, produced no significant difference in red cell ATP, 2,3-DPG, supernatant haemoglobin nor glucose content. There was a change in potassium level in both irradiated and unirradiated samples. The investigators conclude that red cells can be irradiated and stored under the above conditions [165].

In another study on storage of frozen erythrocytes, Miraglia et al. [166] reported that following a radiation dose of 35 Gy, erythrocytes were frozen, then stored for three to 10 months at -80°C prior to transfusion. They found no significant difference in survival between irradiated and unirradiated cells.

Ramirez et al. [167] irradiated red blood cells at 30 Gy, which they then stored for up to 14 days. They found significant increase in the potassium ion content in both irradiated and unirradiated samples of red cells from whole blood and the red cell concentrate. Values following 14 days' storage of whole blood were 31.0 and 15.5 mmol/l for irradiated and unirradiated samples, respectively, and for the red cell concentrate, potassium levels were 68.0 and 31.0 mmol/l for irradiated and unirradiated samples, respectively. They recommend that irradiated blood should not be stored.

In a study by Moore and Ledford [168] on effects of 40 Gy irradiation on the in vitro storage properties of packed red cells, they noted that plasma haemoglobin level was 537 mg/dl in irradiated red cells, and 363 mg/dl in unirradiated samples following 35 days'

storage. After 2 days' storage values were 63 and 60 mg/dl, respectively. ATP and 2,3 DPG were slightly lower in irradiated samples, although the authors did not consider the difference to be clinically significant. There was no difference in pH between irradiated and unirradiated samples. They attributed their findings to the metabolic activity of the red blood cells. They concluded that effects were minimal and from their in vitro data, irradiation does not seem to cause serious damage to red cells.

Results of Button and his colleagues [42] show that 200 Gy irradiation of whole blood and red blood cells, followed by storage for 21 days, had no effect on the survival of the red blood cells. Their findings approached the 19 day biological half life reported by Schiffer et al. [169] following a 350 Gy dose.

3.5.2. The effect of irradiation after storage

Button et al. [43], demonstrated that red blood cells stored for 21 days and then irradiated at 50 Gy, had the same survival as unirradiated controls.

4. PLATELETS

Aggregation and release responses of platelets, and expression of platelet - factor 3 were not influenced by a 50 Gy radiation dose [170]. Similarly, Coifman et al. [112] reported that platelet survival is not appreciably affected by a 50 Gy radiation dose. In fact, gamma irradiation of platelets even at 750 Gy, in vitro, produced no significant damage [171]. In contrast, Button et al. [43] reported that a 50 Gy dose reduced platelet yields. However, transfused irradiated platelets produced the expected increases in platelet counts and controlled haemostasis in thrombocytopenic patients.

Changes in adenine nucleotides of irradiated platelets have been investigated [172]. Human blood platelets were isolated and gamma-irradiated at 5 to 80 Gy. The minimum value for adenosine triphosphate was about 86.3 percent of the control value at a dose of 15 Gy, and the maximum value was about 98.7 percent at 60 Gy. The minimum value for adenosine diphosphate was 87.6 percent at 15 Gy and the maximum value was 111.8 percent at 60 Gy. The minimum value for adenosine monophosphate was 78.5 percent at 15 Gy and the maximum value was about 96.6 percent at 60 Gy [172].

Irradiation of platelets at 100 Gy, followed by 24 h storage at 20 to 24°C, did not induce an increased release of β -thromboglobulin nor enhanced discharge of LDH [173].

Platelets, gamma-irradiated at 15 Gy immediately prior to transfusion, achieved corrected increments within 1 h. Within 20 h of transfusion, their response was not different from that of unirradiated platelets [174].

Guoxin et al. [175], in a study on the effect of Co-60 gamma-ray irradiation on human platelets, found that at 5 Gy, alpha granule membrane protein (GMP 140) molecules expressed on the surface of platelets increased significantly, while glycoproteins Ib and IIIa did not change. At 25 Gy, thromboxane B2 production in plasma was markedly increased. At doses greater than 5 Gy concentration of von Willebrand factor increased with increasing dose. They conclude that platelets can be activated in vitro at doses greater than 5 Gy. In contrast, Stuart has reported that irradiation at 20 Gy does not affect platelet thromboxane B2 production [175a].

The endogenous oxygen uptake by leukocyte-platelet suspensions is reduced by about 20 percent at a radiation dose of 50 Gy. In suspensions of blood platelets, irradiation reduces endogenous respiration, as well as succinic dehydrogenase activity [47]

There was a 10 to 15 percent fall in the platelets count at doses greater than 90 Gy, and following 15 to 17 days' storage after irradiation, this drop had increased to 25 to 30 percent [36].

In summary, it would appear that, excluding lymphocytes, platelets sustain more damage from irradiation than other blood components [38].

4.1. EFFECTS OF STORAGE ON IRRADIATED PLATELETS

Espersen and his colleagues [176] reported no significant difference between platelets irradiated at 15 Gy, then stored at room temperature for 5 days, and unirradiated platelets. However, there was a higher degree of degranulation after 5 days irrespective of whether platelets were irradiated or not. They recommend that irradiation be carried out immediately prior to transfusion.

In a study on the effect of gamma irradiation at 30 Gy within 4 hours of blood collection, followed by storage at 4°C for 42 days, Davey et al. [164] reported no adverse effect on platelet recovery. Similarly, Read et al. [177] investigating the viability of platelets following 30 Gy irradiation and 5 days' storage, showed no significant effect on the in vitro storage characteristics including platelet count, mean platelet volume, pH, and white cell count. In vivo kinetic studies showed a 49.6 percent initial recovery for irradiated platelets (51.3 percent for control) and a mean survival time of 5.6 days (5.9 days for control). They conclude that irradiation does not interfere with the platelets' clinical efficacy.

Investigating the influence of 50 Gy gamma irradiation on platelets that had previously been stored, Moroff et al. [178] found that with 5 days' storage, irradiation caused no change in platelet morphology or ability to undergo synergistic aggregation. Similarly, their other findings for irradiated and unirradiated samples were: mean platelet volumes of 6.82 and 6.83 μm^3 , expression of platelet factor 3 activity of 42.4 and 41.9s, response to hypotonic stress of 0.034 and 0.037 OD² (difference)/min, extent of discharge of LDH of 15.2 and 12.35, release of β -thromboglobulin values of 42.3 and 41.7%, and formation of thromboxane B2 values of 5.80 and 5.13 pg/10⁶ platelets. They conclude that stored platelets are not affected by a radiation dose of 50 Gy. In their study they emphasized that the oxygen tension in the blood containers was 100 torr (mm Hg), indicating full oxygenation of the cells, and that in other studies oxygen pressure may be lower, possibly contributing to hypoxic conditions resulting in greater radiation resistance.

Following a lower radiation dose of 20 Gy, then 5 days' storage, Rock et al. [179] showed that the following parameters of in vitro platelet function were unaffected: platelet count, white cell count, pH, glucose concentration, lactate platelet aggregation, release reaction and serotonin uptake. Even after irradiation of whole blood at 200 Gy, followed by storage for 21 days, no deleterious effect on the survival of platelets was observed [42].

In a study on storage of apheresis platelets following gamma irradiation, Sweeney and his colleagues [180] reported that, irrespective of whether irradiation (25 Gy) was carried out

²OD: Optical density.

one or three days after collection, there was no significant difference in the in vivo recovery or in the survival time, between the irradiated and control samples. Such was the case even after post-irradiation storage for 5 days at 20 to 24°C.

5. PLASMA

Dalos [181] found that the paper electrophoretic pattern of lyophilized irradiated plasma remained unchanged after doses of 20 kGy. After 30 kGy it became somewhat indistinct, and after 100 to 150 kGy entirely confluent. Following starch-gel electrophoresis, they showed a ten percent drop in albumin following 10 kGy radiation dose. Similar findings were reported by Antoni et al. [182] who observed changes in optical absorption in gamma irradiated lyophilized human blood plasma at doses of 50 kGy.

5.1. IRRADIATION OF FROZEN PLASMA

Preliminary investigations by Nisnevitch et al [183], have shown the possibility of cryoradiation sterilization of blood serum and plasma for providing virological and bacteriological safety of blood products and protein blood preparations. They showed that following a 25 kGy gamma-radiation dose, there was less than 10 percent degradation of the fibrinogen content of the plasma, and 1 percent degradation of albumin content. The protein composition of the globulins (alpha 1 and 2, beta and gamma) in the plasma were generally well within 10 percent of the controls. Similar findings were reported for the immunoglobulin content of the cryoirradiated serum. The activity of Factor VIII was at 83 percent. Kitchen et al. [1], based on their investigation of the effect of gamma irradiation on HIV and human coagulation proteins (see below), coupled with the absence of gross changes in other plasma proteins, conclude that irradiation of frozen raw plasma is likely to be highly effective as a means of inactivation of infectious agents present in human plasma [1], while apparently causing minimal deleterious effects on plasma proteins.

In addition, preliminary investigations by Kiselman, Tal'rose, and Trofimov [184] have demonstrated the possibility of irradiation of frozen (cryoirradiation) blood serum and plasma. They have shown the feasibility of such treatment for HIV-positive diagnostic sera for inactivation of HIV viruses without changing serum specific activity.

Irradiation of frozen solutions (cryoirradiation) is not new, and has been investigated in the past with reasonable success in a number of drug systems including cimetidine, ampicillin [185], insulin [186], vitamins [187-189], and liposomes [190].

5.2. BOVINE SERUM

Masefield [191] includes bovine serum in a representative list of gamma radiation sterilized products in the dose range 15 to 50 kGy. Kitchen et al. [1], quoting unpublished data of G.F. Mann, state that gamma-irradiated calf serum contained no substances that were toxic to in vitro culture systems, and that the serum has normal growth promoting characteristics. A study by Lombardo et al. [191a] on irradiation (25 to 50 kGy) of frozen animal serum showed that serum proteins did not suffer alteration.

5.3. EFFECTS ON PROTEINS

5.3.1. Irradiation of frozen solutions

Kitchen et al. [1] undertook a study on the effect of gamma irradiation on human coagulation factors VIII and IX. Irradiation was undertaken in the frozen state at -40°C over the dose range 0 to 40 kGy. Inactivation rates of 0.00173, 0.00526 and 0.00286 \log_{10} units/ml/kGy for Factors VIII:C (procoagulant), VIII:vWF (von Willebrand Factor) and IX, respectively. These values correspond to percentage activity loss rates of approximately 0.4, 0.13 and 0.66%/kGy, respectively.

5.3.2. Irradiation of freeze-dried preparations

Gamma irradiation of lyophilized anti-haemophilic factor (AHF) produced acceptable yields (about 10 percent degradation) with few changes in the electrophoretic migration pattern, following a 10 kGy dose. However, aggregation of albumin was apparent. At 20 kGy there was about 50 percent degradation. It is worth noting that a 10 kGy dose was sufficient to cause 6 log cycles of inactivation of VSV and 3.3 log cycles of inactivation of Sindbis virus [192].

Electron irradiation of freeze-dried powder of bovine fibrinogen caused an approximate 50 percent loss of intrinsic viscosity at 25 kGy [193].

Gamma-irradiation of lyophilized fibrinogen, gamma globulin, and albumin resulted in no appreciable changes in their structure, solubility or chemical characteristics, following doses of up to 30 kGy. Coagulation tests indicated that prothrombin, the Power-Stuart factor, and the Hageman factor, were practically unchanged [194].

5.3.3. Bovine albumin

Bovine albumin fraction V was irradiated by Rainey [30] to rid it of mycoplasma. The powder (with a moisture content of 3 percent) was gamma irradiated with a 25 kGy dose. No gross changes were subsequently observed in viral cells grown in media containing irradiated bovine serum albumin.

Gamma irradiation (40 kGy) in air of solid bovine and human serum albumin caused partial insolubility of the substrate. Irradiation at 80 kGy caused 90 percent of albumin failing to pass through 0.3 μm pores when suspended in water [195].

Irradiation of solid bovine serum albumin in anoxia with 2 MeV electrons, caused a change in water solubility due to increased molecular weight. Similarly, there was a difference in sedimentation behaviour even at doses where average molecular weight was not altered. There was a 20 to 25 percent loss of solubility at 100 kGy, and less than a 5 percent loss of solubility at 25 kGy. There was a 50 percent change in the protein after 50 kGy, and 20 to 30 percent change after a 25 kGy radiation dose [196].

In a subsequent study, again with 2 MeV electrons, over the dose range 10 to 1500 kGy, changes in 9 amino acid residues that constitute over 70 percent of bovine serum albumin were followed. Carbonyl groups were formed, although there was no evidence of breaks in the main chain. Alexander and Hamilton [197] reasoned that the changes in amino acid residues could not explain the observed denaturation. The changes were insignificant at less

than 50 kGy [197]. Carroll et al. [198] presented evidence for extensive polymerization of bovine serum albumin, but no signs of degradation following X-irradiation of air saturated solutions at 200 Gy to 15 kGy. Sensitivity increased at the iso-electric point and below pH5.3.

X-irradiation of aqueous solutions of bovine serum albumin, and bovine serum gamma-globulin caused an increase in absorbance with increasing radiation dose. The radiation effect was insignificant at 10 Gy. Precipitation was evident when a 0.07 percent aqueous solution of serum albumin was irradiated with 750 Gy at 25°C. No precipitation occurred when solutions were in ice or at higher concentrations. There was a rise in viscosity at 500 Gy. Dimerization occurred at 1 kGy [199].

5.3.4. Human albumin

X-irradiation of a 0.1 percent salt-free iso-electric solution of human serum albumin with a 205 Gy dose produced no change in the UV spectrum [200].

Gamma-irradiation of human serum albumin aqueous solutions (1 percent), caused a 50 percent decrease in potency as measured by sedimentation profiles, following a 2 kGy dose. The addition of sodium benzoate (7 mM) reduced the degradation to less than 5 percent [201].

De Alva and Cortina [194] reported that gamma irradiation of albumin at doses of up to 30 kGy resulted in no appreciable changes in structure or chemical characteristics.

5.3.5. Immunoglobulins

Freeze dried human immunoglobulin G (IgG) retained its reactivity after gamma-irradiation with doses of 15 to 25 kGy. However, gel-filtration indicated the presence of aggregated IgG in addition to the non-aggregated form. Proteolytic experiments revealed an altered digestibility of the protein with papain after irradiation [202].

6. STORAGE OF WHOLE BLOOD

Effects of storage of individual blood components have been discussed earlier. In studying the effect of storage of whole blood, Ramirez et al. [167] irradiated whole blood at 30 Gy, which they then stored for up to 14 days. Plasma haemoglobin content was not significantly different between irradiated and control samples following 5 days' storage, but the difference was significant after 14 days. They recommend that irradiated blood not be stored.

7. HEPARIN

Heparin is often used as an anticoagulant for blood samples. It shows a loss of activity when irradiated in aqueous solution [19]. This finding, confirmed by Blackburn et al. [27], is attributed to reaction with .OH to form acids and reducing products. Loss of dye-binding capacity [203] and anticoagulant properties [204] accompanies these chemical changes.

8. PLASTICIZERS

The possible toxic effects of plasticizer, present in blood unit bags and received from transfusions of blood and blood products, have been of concern, particularly in the neonate. This concern is coupled with the possibility that irradiation could potentiate the leakage of plasticizer into these products [205-206].

9. ECONOMIC ASPECTS OF BLOOD IRRADIATION

According to Anderson et al. [109], the financial implications of blood irradiation are considerable. Bearing in mind that only 12 percent of the US institutions which they surveyed possessed irradiation facilities, a regional approach would appear prudent. They estimate costs at \$50 000 for a caesium-137 source, in addition to labour costs.

An editorial by Leitman [111] states that ninety new orders for blood irradiators were placed in 1992, and the number of free-standing irradiators in the USA alone is approximately 400 (1993). Their use has moved into the arena of every day transfusion care.

Following a 1989 survey by the American Association of Blood Banks, of 452 US institutions giving neonatal transfusions, it was reported [115-116] that in 13 percent of the surveyed institutions, gamma irradiated blood components were given to all patients. In 46 percent of the institutions, irradiated blood components were only given to some of the neonates to prevent graft-versus-host disease, and in 41 percent of the institutions no irradiation of any of the blood products was carried out.

10. PRACTICAL ASPECTS

10.1. TECHNICAL DIFFICULTIES IN BLOOD IRRADIATION

The major technical problems in blood irradiation are guaranteeing dose homogeneity, erratic turntables within the irradiation chamber, and errors in calculating the radioactive decay of the source (either caesium-137 or cobalt-60) [111, 206a]. In order to overcome some of these problems, the FDA, in a draft memorandum on blood irradiation, will require verification of the recommended dose of 25 Gy to the mid-plane of the irradiated blood unit, and that a minimum dose of 15 Gy is delivered throughout the unit [207].

The ASTM (USA) Subcommittee E10.01 [208] concerned with standards for dosimetry for radiation processing, has very recently set up a new task group to prepare a standard practice for dosimetric procedures for irradiation of blood and blood products.

The necessity for quality assurance in the irradiation of blood components was emphasised at an American Association of Blood Banks' workshop, held in 1992, and devoted to this topic [209].

10.2. USE OF RADIOTHERAPY FACILITIES

The use of radiotherapy facilities for blood irradiation is not generally recommended because of the inconvenience and uncertainty in the delivered dose, with self contained units

being the better choice, particularly those with a caesium-137 radiation source with its long half life. Caesium-137 units are expensive but maintenance is easy, and irradiation treatment is completed within a few minutes [95]. Use of commercially available Cs-137 sources is, according to Leitman and Holland [86], the most commonly used method of irradiating blood packs.

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