# Storage of irradiated human blood; a source of error in quantitative chromosome analysis

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*Summary.* Human whole blood was irradiated with 2.5 Gy of 220 kVp X-rays and stored before culture with 9.7 μM BrdU and 19.4 or 38.7 μM BrdU for 0, 24, 48 and 72 h. The frequency of dicentrics and ring chromosomes was determined in cells staining as first division (M1) metaphases with the fluorescence plus Giemsa technique. Storage had no influence on the observed aberration yields in 44 h cultures containing 9.7 μM BrdU. In 66 h cultures at 19.4 μM BrdU the observed yields after 2 and 3 days' storage were significantly lower as compared to cultures from fresh blood. No storage effect was revealed in 66 h cultures containing 38.7 μM BrdU. In cases where cytogenetic radiation dosimetry has to be carried out using blood samples which have been in transit for 2–3 days, the findings are of relevance for a correct determination of the chromosome damage in M1 cells.

*Key words.* Human lymphocytes; X-irradiations; blood storage; chromosome aberrations; fluorescence plus Giemsa staining; cytogenetic dosimetry.

For any quantitative analysis of radiation-induced chromosome aberrations the influence of cell cycle kinetics has to be considered. This is of special importance for a cytogenetic dose estimation after radiation exposure. Since aberrations can be eliminated during successive cell divisions dose estimates can be misleading in any analysis which does not use exclusively first division cells (M1). After treatment of lymphocyte cultures with bromodeoxyuridine (BrdU) metaphase cells that have replicated for one, two (M2) or three (M3) cycles can be unequivocally identified with the FPG-staining technique (fluorescence plus Giemsa)[1].

It has been demonstrated previously[2] that for this reason it is necessary to determine a concentration of BrdU that has no inhibitory effect on cell proliferation but allows a distinct harlequin-staining (M2, M3 cells) for a specific sampling time and culture condition.

Recently Prosser and Moquet[3] reported that in cultures set up from stored blood many cells failed to stain differentially at BrdU concentrations of 10 or 20 μM, whereas at 40 μM a correct identification of M1 and M2 cells could be achieved.

When blood samples from radiation-exposed persons are distributed to cytogenetic laboratories this usually involves a delay of some days. When blood cultures cannot be established with fresh blood, this will have immediate consequences for an exact cytogenetic radiation dosimetry in cases where the BrdU concentrations used are too low. An influence of the effect of blood storage on differential staining of human lymphocytes should be directly reflected in the incidence of radiation-induced chromosome aberrations. This was analysed in the present study.

*Material and methods.* Whole blood of a healthy female donor was irradiated with 2.5 Gy of 220 kVp X-rays at 37 °C (filter 4.05 mm Al + 0.5 mm Cu; dose rate 0.5 Gy · $min^{-1}$). From irradiated whole blood two replicate cultures were set up after storage of blood samples for 0, 24, 48 and 72 h at 19 °C in the dark. The BrdU concentration in 44-h cultures was 9.7 μM, in 66-h cultures 19.4 of 38.7 μM. A detailed description of culture procedures and FPG-staining is given elsewhere[2,4].

The frequency of dicentrics + ring chromosomes ($R_c$) was determined in each of 12 culture categories in metaphases staining as M1. For the determination of significant differences between categories mean values of aberration yields were tested for homogeneity taking a probability of 0.05 as the level of significance[5].

*Results and discussion.* The table shows the mean values of dicentrics + $R_c$ per cell for blood cultures with different BrdU concentrations and established after 0, 24, 48 and 72 h storage of irradiated blood. The data from 44-h cultures containing 9.7 μM BrdU (our standard conditions for fresh blood) reveal no significant differences for the aberration yields observed after the different storage periods.

As we could demonstrate in a previous study[2], the frequency of harlequin-stained cells in 48-h control cultures ranged between 24 and 64% for various donors. Although in the present experiment only M1 cells were found, the aberration yields were not significantly different after the different storage periods. This suggests that because of the shorter culture time and the radiation-induced mitotic delay the chromosome damage was correctly identified in true M1 cells.

In 66-h cultures, where usually a high proportion of M2 cells has to be expected, Prosser and Moquet[3] observed an increase in the percentage of cells staining as M1 with increasing storage time when they used 10 or 20 μM BrdU. It was suggested that in contrast to cultures established from fresh blood, storage prevents an adequate differential staining (harlequinization) of M2 metaphases at BrdU concentrations below 20 μM, owing to a failure in differential uptake of Giemsa stain. At 40 μM BrdU a reliable identification of M1 and M2 cells could be achieved. This is in accordance with our earlier findings[2] with cultures of fresh blood, where the BrdU concentration had to be increased with increasing culture time in order to obtain distinct harlequin staining.

The present data from experiments with irradiated blood (table) confirm these results. As compared to cultures established with fresh blood, containing 19.4 μM BrdU, storage for 48 and 72 h resulted in significantly lower aberration yields. Storage for 24 h still had no influence. No storage effect was revealed in 66-h cultures with 38.7 μM BrdU, where the aberration yields were not significantly different from those observed in 44-h cultures with only M1 cells.

Our data clearly indicate that in 66-h cultures 19.4 μM BrdU is insufficient to guarantee a reliable differentiation of M2 cells in cultures from blood stored for 2–3 days. Obviously in these cultures M2 cells which stained like M1 cells must have been included in the chromosome analysis, which explains the lower aberration yields. Similar, by as reported earlier[3], the quality of FPG-staining was reduced in our 66-h cultures especially at the lower BrdU concentration. For a correct interpretation of the

Dependence of the frequency of dicentrics + ring chromosomes on blood storage and culture conditions

| Storage time (h) | BrdU (μM) | Culture time (h) | Cells scored | Dicentrics + $R_c$ per cell ± SE |
|---|---|---|---|---|
| 0 | 9.7 | 44 | 500 | 0.51 ± 0.03 |
| 24 | 9.7 | 44 | 500 | 0.55 ± 0.03 |
| 48 | 9.7 | 44 | 500 | 0.54 ± 0.03 |
| 72 | 9.7 | 44 | 500 | 0.54 ± 0.03 |
| 0 | 19.4 | 66 | 352 | 0.48 ± 0.04 |
| 24 | 19.4 | 66 | 424 | 0.47 ± 0.03 |
| 48 | 19.4 | 66 | 294 | 0.40 ± 0.06 |
| 72 | 19.4 | 66 | 500 | 0.37 ± 0.03 |
| 0 | 38.7 | 66 | 500 | 0.55 ± 0.03 |
| 24 | 38.7 | 66 | 466 | 0.49 ± 0.03 |
| 48 | 38.7 | 66 | 429 | 0.51 ± 0.03 |
| 72 | 38.7 | 66 | 437 | 0.56 ± 0.04 |

chromosome aberration findings many cells had to be rejected, which explains why a total cell number of 500 could not always be analysed.

*Conclusions.* An exact quantification of radiation-induced chromosome aberrations in human lymphocytes requires the analysis of M1 cells. In blood cultures with a high proportion of M2 cells established after storage of blood for 2 or 3 days a correct identification of M1 and M2 cells is impossible when only 19.4 µM BrdU is used for the FPG staining method. As a consequence the actual chromosome damage in M1 is significantly underestimated. When culture initiation is delayed for more than 24 h and 38.7 µM BrdU is added to the cultures, M1 and M2 cells can be reliably identified and the chromosome damage can be correctly analysed. This should be taken into account for cytogenetic radiation dosimetry purposes when mailing of blood samples, causing a delay of several days, occurs.

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## A mechanism for macromolecular transfer from glia to neuron cell body in crayfish

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*Summary.* Small extrusions of glial cytoplasm are endocytosed by neuron cell bodies of the crayfish *Procambarus*. Vesicles are double walled with the external membrane issuing from the neuron and the internal one from the glia. This could be a system for the transfer of glial cytoplasmic free proteins to neurons.

*Key words.* Crayfish; double-walled vesicles; glia; modified endocytosis; motoneurons.

Glial cells can transfer proteins to the axons of invertebrates[1-4]. Several structures, such as tubular lattices[5,6] and trans-glial channels[7,8] could be concerned with glia-to-axon transfer. Glial exocytosis coupled to axonal endocytosis could also account for the transfer of proteins from glial vesicles through the intercellular space[4]. Nevertheless, no mechanism has yet been described for the glia-to-axon transfer of cytoplasmic free proteins such as actin[4]. Junctional structures for the glia-to-neuron cell body transfer of free cytoplasmic proteins or vesicular macromolecules have not been reported. Moreover, neuron somata of both vertebrates and invertebrates are separated from glia by a space of 10–20 nm which is considered as the microenvironment of the nervous cells, i.e. the site for ionic and macromolecular glia-neuron exchange[1,9,10]. In crayfish, however, gap-like junctions have been reported[11]. Modified endocytosis of small glial cytoplasmic extrusions by abdominal neuron cell bodies of crayfish is described here. It could be involved in glia-to-neuron cell body transfer.

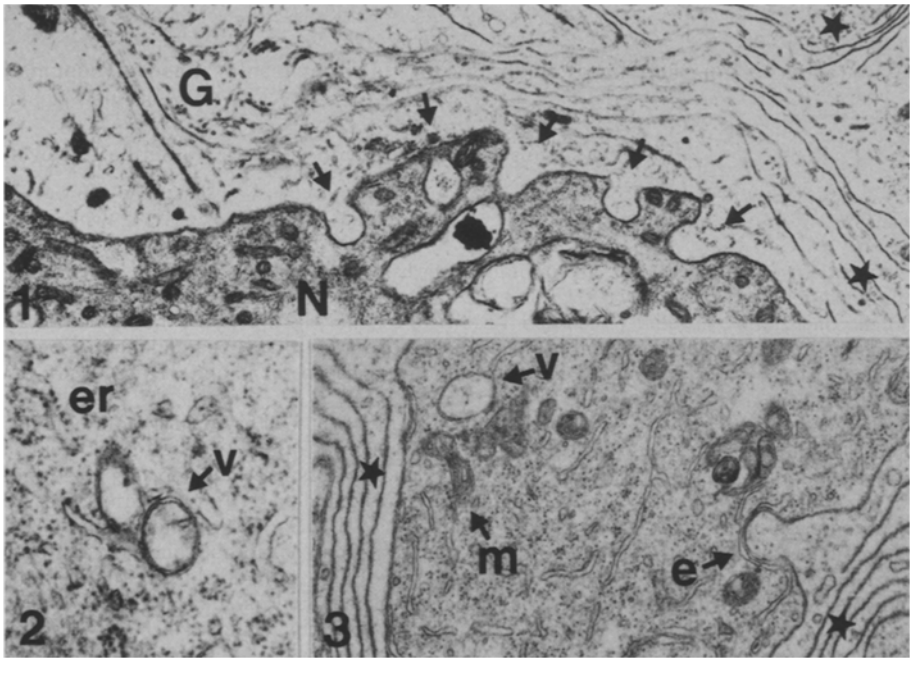


Figure 1. Modified neuronal endocytosis of glial cytoplasmic extrusions. Vesicles (arrows) are shown at different stages of the process. Note the difference in electron density between glial (G) and neuronal (N) cytoplasms. Asterisks: membraneous systems of the glial cells ( × 11,540).

Figure 2. A double-walled vesicle (v) with light content within a neuron cell body. er, rough endoplasmic reticulum ( × 28,900).

Figure 3. Detail of a neuron cell body covered by membraneous processes of perineuronal glial cells (asterisks). e, double-walled endocytotoc figure, m, mitochondrion. v, double-walled vesicle with light content ( × 16,250).