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● *Chromosome Analysis*

NOVEL BIODOSIMETRY METHODS APPLIED TO VICTIMS OF THE GOIÂNIA ACCIDENT

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***Abstract*—Two biodosimetric methods under development at the Lawrence Livermore National Laboratory were applied to five persons accidentally exposed to a ^{137}Cs source in Goiânia, Brazil. The methods used were somatic "null" mutations at the glycophorin A locus detected as missing proteins on the surface of blood erythrocytes and chromosome translocations in blood lymphocytes detected using fluorescence *in-situ* hybridization. Biodosimetric results obtained ~1 y after the accident using these new and largely unvalidated methods are in general agreement with results obtained immediately after the accident using dicentric chromosome aberrations. Additional follow-up of Goiânia accident victims will 1) help provide the information needed to validate these new methods for use in biodosimetry and 2) provide independent estimates of dose.**

INTRODUCTION

TWO NEW biodosimetry methods under development at the Lawrence Livermore National Laboratory for the past few years have been applied to five individuals involved in the Goiânia accident. These methods are 1) measurement of somatic "null" mutations involving the glycophorin A (GPA) gene in bone marrow stem cells detected as a missing GPA allelic protein on the surface of peripheral blood erythrocytes (Langlois et al. 1986, 1990) and 2) measurement of chromosome translocations detected using fluorescence *in-situ* hybridization of chromosome-specific DNA probes (Pinkel et al. 1986; Lucas et al. 1989a, 1989b). To validate these new methods for use in biodosimetry, GPA analyses have been made of blood from Hiroshima A-bomb survivors (Langlois et al. 1987) and chemotherapy patients exposed to chemical mutagens (Bigbee et al. 1990), and human blood lymphocytes have been used to measure dose-response curves for radiation-induced chromosome translocations *in vitro*. These previous studies, together with available data from the published literature (e.g., Awa et al. 1978; Buckton et al. 1978), show that both GPA mutations and translocations are persistent indicators of biological damage. For example, the GPA assay was used to reconstruct a dose-response curve for Hiroshima A-bomb survivors exposed more than 40 y previously. However, these assays require further validation before they can be reliably applied to biodosimetry.

The Goiânia accident occurred in September 1987 and involved a ^{137}Cs irradiator containing a 50.9-TBq (1375-Ci) source that was dismantled and handled by persons unaware of the dangers (Ramalho et al. 1988; Ramalho and Nascimento, this issue). Initial studies of the frequencies of dicentric chromosomes in lymphocytes indicated that over 100 persons were exposed in the Goiânia accident to doses ranging from near background to more than 7 Gy (Ramalho et al. 1988). Thus, this accident affords the opportunity to compare the responses of the two new assays to established cytogenetic methods in individuals exposed to a broad range of radiation doses. It is also significant that because biodosimetric measurements began immediately after the accident for dicentrics and about 1 y later for GPA and translocations, the temporal stability of these biodosimeters can be evaluated through additional follow-up of the same individuals.

MATERIALS AND METHODS

Blood samples

Peripheral blood was obtained from accident victims during medical follow-up in Brazil. Each sample consisted of 10 mL of blood drawn into a heparinized vacutainer. The sample was coded with an identification number, placed in an insulated box, and shipped on ice for GPA and translocation analyses to the Lawrence Livermore National Laboratory (LLNL). The blood was received at LLNL within about 3 d of collection.

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At LLNL, the blood was first serotyped using commercial anti-M and anti-N sera.[‡] Since the GPA assay requires blood of heterozygous MN type, the samples that were homozygous (MM or NN) were discarded. The MN samples were then divided into two aliquots, one for GPA assay and the other for translocation analysis. The blood used for GPA assay was fixed, labeled with monoclonal antibodies, and analyzed within 3 d of labeling on a flow cytometer (Langlois et al. 1990). The blood for translocation analysis was processed to separate lymphocytes from whole blood by centrifugation.

Following the analyses, the samples were decoded; subject ID numbers used in this paper match those used in the cytogenetics paper by Ramalho and Nascimento (this issue), and therefore permit intercomparisons of results for specific individuals. To date, we have received blood samples from subjects 1 and 8 on two separate occasions, first in September 1988 and another in April 1989. Subjects 13 and 14 were sampled only in September 1988 and subject 51 in April 1989.

GPA assay

The GPA assay uses immunofluorescence labeling and flow cytometry to enumerate the frequency of red blood cells that lack expression of one allelic form of this protein due to mutations in erythroid precursor cells (Langlois et al. 1986). The GPA gene is located on human chromosome 4 and is present in the population as two common alleles at approximately equal frequencies so that ~50% of the population is heterozygous. The GPA proteins coded for by these two alleles are referred to as M and N and differ by only two amino acids out of a total of 131. GPA is located on the red blood cell surface, which facilitates its detection using monoclonal antibodies.

While the GPA assay was originally developed using a dual-laser flow sorter (Langlois et al. 1986), the data in this paper were obtained with a modified method (designated the BR6 assay) performed on a simpler flow cytometer (Langlois et al. 1990). The assay enumerates the frequency of "null" mutation variant cells (N∅) in the blood of MN heterozygotes, i.e., cells that have lost expression of the M allele but express the N allele normally. In 17 normal unexposed persons studied at LLNL using the BR6 assay, the N∅ frequency ranges from 3.0 to 12.7 variants per million cells (median, 5.1; mean ± 1 SD, 6.1 ± 2.6) (Langlois et al. 1990).

Translocation analysis

The method used to detect chromosome translocations was a fluorescence *in-situ* hybridization-based approach recently developed at LLNL. This method has been described previously (Pinkel et al. 1986; Lucas et al. 1989a, 1989b). Briefly, separated lymphocytes were stimulated to proliferate with 0.15 mg mL^{-1} phytohemagglutinin (PHA). After 48 h, colcemid was added to each culture, and 4 h later metaphase spreads were prepared on glass slides by the method of Evans et al. (1971). DNA in the metaphase chromosomes was denatured at 70°C, dehydrated, and air-dried. The biotin-labeled DNA probes were also thermally denatured at 70°C, added to the slides, and incubated overnight. (For additional information on chromosome-specific DNA probes, see Lucas et al. 1989b.)

Bound probes were detected by treating sequentially with avidin conjugated to fluorescein, a monoclonal antibody against avidin labeled with biotin, and finally a second layer of avidin conjugated to fluorescein. This results in essentially uniform painting along the entire lengths of the targeted chromosomes with a bright fluorescent green-yellow color. With subsequent propidium iodide staining, all other chromosomes not specifically labelled are made to fluoresce red. The final result is metaphase spreads having selected chromosomes fluorescing bright green-yellow and the other chromosomes fluorescing red. Translocation of material between red and green-yellow chromosomes is very easy to detect using fluorescence microscopy.

Fluorescence *in-situ* hybridization was performed using two cocktails of composite DNA probes, cocktail A is specific to chromosomes 1, 2, and 4, and cocktail B is specific to chromosomes 1, 3, and 4. Cocktail B was used for subjects 1 and 51, and cocktail A for subject 8. The use of two different cocktails had no special significance other than the availability of probes.

In order to compare our translocation results using this approach with those from conventional cytogenetic methods, we scale our smaller target up to equal the full genome. The formula used to scale to the full genome has been described previously (Lucas et al. 1989c):

$$F_T = F_H/2f_s(1 - f_s), \qquad (1)$$

where the translocation frequency detected by fluorescence hybridization is F_H, the full genomic translocation frequency is F_T, and f_s is the fraction of the genome hybridized. The fraction of the total genomic DNA contained in chromosomes 1, 2, 3, and 4 is 0.0864, 0.0844, 0.0698, and 0.0668, respectively (Mendelsohn et al. 1973). Hence, the f_s for cocktail A is 0.238, and the f_s for cocktail B is 0.223.

RESULTS

Table 1 lists GPA results for five persons known or suspected of having been exposed in the Goiânia accident. The values listed are the mean N∅ variant cell frequencies obtained from duplicate measurements of repeat samples obtained in September 1988 and April 1989, and standard errors of the means based on the variability between measurements. The observed variant frequencies for these Goiânia accident victims exceed the highest frequency observed in samples from 17 normal unexposed donors, except for subject 51 who is in the normal range.

The ranking order from highest to lowest GPA variant cell frequency is subject 1, 8, 13, 14, and 51. This ranking order is the same as that obtained for dicentrics

[‡] Ortho Diagnostics, Raritan, NJ.

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Table 1. GPA assay results for Goiânia accident victims.

Subject ID	NØ variants per 10^6 cells[a]	SE[b]
1	91.3	21.5
8	47.0	7.6
13	15.5	2.5
14	13.0	1.0
51	5.5	3.5

[a] Mean of duplicate measurements obtained at 1 and 1.4 y postexposure.
[b] Standard error of the mean obtained from variations between measurements.

(Table 2). Interestingly, the quantitative comparison between GPA and dicentric frequencies (Fig. 1) shows an excellent linear correlation between these two endpoints (see Discussion).

Table 2 lists cytogenetic results. Translocations are those obtained here using the fluorescence hybridization method discussed above and have been scaled to the full genome. The translocation frequency (translocations per cell) for subject 1 is the mean from repeat samples obtained in September 1988 and April 1989, and those for subjects 8 and 51 are from single samples obtained in April 1989 and September 1988, respectively. Also listed are fractions of cells with translocations. The results for dicentrics plus rings are those of Ramalho et al. (1988) obtained in October 1987, immediately after the accident. Dicentrics plus rings were also scored in blood samples obtained ~1 y later from subjects 1 and 8 for GPA and translocation analyses. Results for Subject 51 are reported here only.

For translocations, we have results only from subjects 1, 8, and 51. Again, the highest frequency is seen in subjects 1 and 8, and the lowest in subject 51. No translocations were observed in 1043 cells from subject 51, consistent with results obtained for unexposed controls in our laboratory.

Biodosimetric results are given in the Discussion and Table 3.

DISCUSSION

Dose estimation for the subjects involved in the Goiânia accident using results for dicentrics obtained immediately after the accident has been performed elsewhere (Ramalho et al. 1988; Ramalho and Nascimento, this issue; Littlefield and Lushbaugh 1990). The purpose here is not to estimate the *actual* doses, but rather, to intercompare results for the same individuals using different biodosimetric methods. Thus, for our purposes, consideration need not be made of dose rate. The comparisons in this paper are therefore simplified by the use of standard curves for acute exposures for all three endpoints, i.e., GPA, translocations, and dicentrics.

Listed in Table 3 are dosimetry results for five Goiânia accident victims, obtained for acute-equivalent exposures. Dose estimates determined from our GPA and translocation results obtained 1 to 1.4 y after the accident are compared with those determined using results for dicentrics plus rings from Ramalho et al. (1988) and for dicentrics from Littlefield and Lushbaugh (1990) obtained immediately after the accident.

The acute-equivalent dose estimates listed in Table 3 for dicentrics and for dicentrics plus rings were obtained using linear (α) and quadratic (β) coefficients from *in vitro* studies and solving for dose, D (Gy), in the linear-quadratic equation. For example:

$$Y_{dic} = \gamma + \alpha D + \beta D^2, \qquad (2)$$

where Y_{dic} is the number of dicentrics per cell measured in lymphocytes of Goiânia accident victims within 1 mo

Table 2. Cytogenetic results for Goiânia accident victims.

Subject ID	Fluorescence hybridization[a]	Conventional cytogenetics[b]	
		Dicentrics plus rings per cell	
	Translocations per cell	$t < 1$ mo	$t = 1$ y
1	0.26 ± 0.07[c]	1.25 ± 0.09	0.24 ± 0.05
8	0.35 ± 0.09	0.53 ± 0.08	0.11 ± 0.06
13	—[d]	0.16 ± 0.07	0.015 ± 0.017
14	—[d]	0.10 ± 0.03	—[e]
51	0	0	—[e]

[a] From this paper; blood obtained 1 to 1.4 y after the accident. Results have been scaled to the total genome using eqn (1). Subjects 1, 8, and 51 had 15 translocations per 165 cells, 17 translocations per 134 cells, and 0 translocations per 1043 cells, respectively. Subject 1 had two cells with two translocations each.
[b] Blood obtained immediately after the accident and also at ~1 y after the accident (Ramalho et al. 1988; Ramalho and Nascimento, this issue). Results for subject 51 are reported here only.
[c] Mean ± SE. Values of SE for translocations and dicentrics were obtained from the data assuming Poisson statistics.
[d] Not scored due to poor growth of lymphocytes in culture, probably due to the long transportation time.
[e] Data from Ramalho and Nascimento (this issue) not available.

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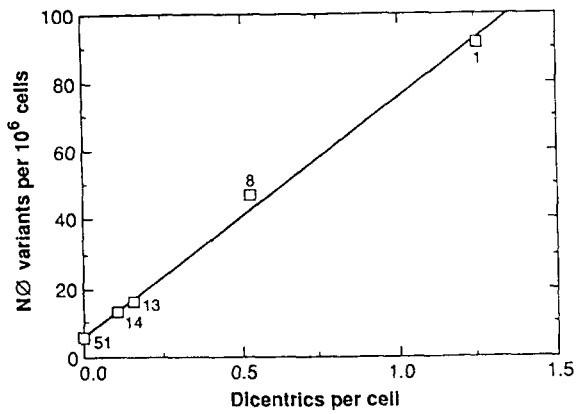

Fig. 1. GPA variant cell frequencies for Goiânia accident victims plotted against their chromosome dicentric frequencies [dicentric plus ring frequencies obtained immediately after the accident by Ramalho et al. (1988); see Table 2]. Subject ID numbers are indicated. The line corresponds to a linear regression on these data; the y-intercept is 6.2 NØ variants per million cells, and the slope is 69.1 NØ variants per million cells/dicentric plus ring per cell.

after the exposure, γ is the background frequency that is typically less than ~0.001 dicentric per cell (Lloyd et al. 1980), and α is 1.57×10^{-2} dicentric per cell per Gy and β is 5.70×10^{-2} dicentric per cell per Gy^2 for acute ^{60}Co γ-ray exposure of human blood lymphocytes *in vitro*. (For dicentrics plus rings, α and β coefficients are 1.99×10^{-2} and 6.4×10^{-2}, respectively, Littlefield and Lushbaugh 1990.) Use of such coefficients from *in-vitro* calibration curves to estimate the dose to exposed individuals is justified by the observation that identical aberration frequencies are induced in lymphocytes *in vitro* and *in vivo* (e.g., Bajerska and Liniecki 1975; Brewen and Gengozian 1971). As mentioned above, because our aim here is not to reconstruct the actual doses but rather to compare the various biodosimetry methods, standard curves for acute exposure have been used throughout this paper for uniformity. The doses listed in Table 3 for dicentrics or dicentrics plus rings were obtained from eqn (2) using appropriate dose-response coefficients and data obtained immediately after the accident by Ramalho et al. (1988) and by Littlefield and Lushbaugh (1990).

For GPA, we used two different approaches to estimate dose (see Table 3). The first approach (Approach 1) was to use the linear dose-response relation derived from previous studies of the Hiroshima A-bomb survivors to convert NØ mutation frequencies (Y_{GPA}) measured in Goiânia accident victims into acute-equivalent doses. That is, solving for D in the linear equation:

$$Y_{GPA} = \gamma' + \alpha' D, \tag{3}$$

where γ' is the mean background NØ variant frequency (6.1×10^{-6} per cell from Langlois et al. 1990), and α' is the linear coefficient (22×10^{-6} per cell per Gy) obtained from Hiroshima GPA data of Langlois et al. (1987).

The second approach (Approach 2) was to use a linear-quadratic dose-response model for GPA frequencies. The excellent linear fit in Fig. 1 suggests that the shapes of the dose-response curves for GPA and dicentrics are similar. Since the curve for dicentrics is linear-quadratic (e.g., Littlefield and Lushbaugh 1990), this would imply that GPA also has this shape. To estimate the linear-quadratic dose-response function for GPA mutations, we simply multiply the slope (69.1×10^{-6} NØ variants per cell/dicentric per cell) of the curve in Fig. 1 with the linear (0.0157 dicentric per cell per Gy) and the quadratic (0.057 dicentric per cell per Gy^2) coefficients for dicentrics from Littlefield and Lushbaugh (1990). This results in linear and quadratic coefficients for GPA mutations as shown in eqn (4):

$$\text{NØ variants per cell} = 6.2 \times 10^{-6} + 1.09 \times 10^{-6} D + 3.94 \times 10^{-6} D^2. \tag{4}$$

Here, 6.2×10^{-6} is the estimated background frequency that is the y-intercept of the curve in Fig. 1. (This is fortuitously nearly identical to the mean background frequency used in eqn 3.)

The results for both Approach 1 and 2 are given in Table 3. It is noted that dose estimates for Approach 1

Table 3. Biodosimetric intercomparisons of GPA and cytogenetic endpoints for Goiânia accident victims.[a]

Subject ID	GPA (this paper)[b]		Translocations (this paper)[b]	Dicentrics (Littlefield)[c]	Dicentrics plus rings (Ramalho)[d]
	Approach 1	Approach 2			
1	3.9	4.5	2.0	3.8	4.3
8	1.9	3.1	2.3	—	2.7
13	0.4	1.4	—	0.7	1.4
14	0.3	1.2	—	—	1.1
51	~0	~0	~0	—	~0

[a] Acute-equivalent doses in Gy; these are not the actual doses that resulted from chronic or subacute exposures, but are used here for method intercomparison purposes.
[b] Based on GPA and translocation frequencies at 1 to 1.4 y after the accident.
[c] Based on dicentric frequencies immediately after the accident; from Littlefield and Lushbaugh (1990).
[d] Derived from dicentric plus ring frequencies obtained immediately after the accident by Ramalho et al. (1988, Table 2) using appropriate coefficients for acute ^{60}Co γ rays from Littlefield and Lushbaugh (1990) in eqn (2).

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are substantially lower at the lowest doses than those for Approach 2, and Approach 2 provides a much better agreement with the results for dicentrics. These differences are primarily due to the shapes of the dose-response curves used as standard. A linear standard curve (used in Approach 1) will always produce lower dose estimates at low to moderate doses than a linear-quadratic curve (used in Approach 2). The striking similarity between dose estimates in Table 3 for GPA (Approach 2) and dicentrics results from the excellent correlation of the data in Fig. 1 and the use of linear-quadratic standard curves for both GPA and dicentrics.

Dose estimates are also affected by the assumed background frequency. Since pre-exposure N∅ frequencies were of course not obtained for the Goiânia accident victims (nor for Hiroshima survivors), individual background frequencies must be inferred from other data and therefore represent an additional source of uncertainty. Like curve shape, uncertainties in the background will also primarily affect the estimates at low to moderate doses. As discussed above, the mean N∅ frequency for 17 normal unexposed individuals was 6.1×10^{-6} variants per cell. This is very similar to the measured frequency of 5.5×10^{-6} in Subject 51 who does not appear to have received detectable exposure (as judged by the cytogenetic endpoints) and is also nearly identical to the y-intercept in Fig. 1. These results would suggest that uncertainties in the background frequencies for individual accident victims may be less important than uncertainties in the shape and slope of the dose-response curve.

For translocations, the target DNA was scaled to the full genome [using eqn (1) and data given above]. It should be noted that the frequency of translocations observed in peripheral lymphocytes more than a couple of months after exposure will depend upon the rate of elimination of the mature irradiated lymphocytes from the circulation as a result of normal attrition, as well as upon the rate of repopulation of the peripheral blood compartment by progeny of irradiated stem cells that may also contain "stable" types of chromosomal rearrangements. Presently, adequate models are not available to predict the complex cellular dynamics that affect observed translocation frequencies in mature lymphocytes sampled at various times after exposure (i.e., the effects of time interval, total dose, fraction of mature lymphocytes, and irradiated lymphocyte progenitors that sustained chromosome aberrations of the stable as well as unstable type, etc.). Thus, our present data for translocations can be used to obtain only crude estimates of dose at this time.

The dose estimates listed in Table 3 for translocations were obtained by simply substituting the translocation frequencies measured at approximately 1-y postexposure (from Table 2), with Y_{dic} in eqn (2), and solving for D. These calculations assume that dicentrics and translocations are induced with the same frequency at the time of exposure (e.g., Brewen and Preston 1975), and further assume that translocation frequencies remain "stable" and do not change with time postexposure, as has been suggested in long-term follow-up studies of patients having fractionated partial-body radiotherapy (Buckton et al. 1978). Our present observations (Table 2) indicate that the translocation frequencies obtained at 1 y in these radiation accident victims are indeed lower than the dicentric frequencies obtained immediately after the accident. Thus, it appears that the dose estimates based on translocation frequencies at 1 y represent underestimates of the doses actually received at the time of exposure.

Given the present uncertainties and small sample size, the results indicate a general agreement between the different methods used as well as the different laboratories. Subjects 1 and 8 are highest and subject 51 is the lowest for all assays. For the reasons discussed above, the results for translocations for subjects 1 and 8 are uncertain. The agreement between Approach 2 for GPA and dicentrics is impressive and suggests that the dose-response curve for GPA is linear-quadratic, similar to that for dicentrics.

Further follow-up of persons exposed in the Goiânia accident should provide additional insights into the dose and time responses of these novel biodosimetric methods and will help validate them for use in biodosimetry.

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