Biodosimetry: chromosome aberration in lymphocytes and electron paramagnetic resonance in tooth enamel from atomic bomb survivors

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Introduction
An important task in studying the health effects of atomic bomb radiation is the estimation of individual doses. Simple comparison of those who were exposed to the bombs within 2.5 km of the hypocentre (i.e., those who potentially received high doses) and those exposed beyond 3.5 km (i.e., practically an unexposed group) is not only inefficient for detecting radiation effects (i.e., among those exposed to substantial doses, only about a two-fold increase in mortality from leukaemia and a 10% increase in other cancers has been observed) but is also uninformative for evaluating risk in other radiation-exposed populations because no common scale of reference exists.

In the follow-up studies of the atomic bomb survivors conducted by the Atomic Bomb Casualty Commission – Radiation Effects Research Foundation (RERF), individual doses have been estimated on the basis of recalled location and shielding conditions at the time of the bombings. This information had been collected by interviewing survivors more than 5 years after the bombings. For this purpose, Japanese houses were replicated at the Nevada Test Site to directly measure absorption by roof tiles or mud walls ("Ichiban" project) (1). In 1965, doses were assigned to about 90,000 survivors (known as the tentative 1965 dosimetry or Tu65D). In 1986, a new dosimetry system, termed Dosimetry System 1986 or DS86, was introduced to estimate individual doses. Physical dose measurements of roof tiles, etc., fit reasonably well with the DS86 dose estimates as far as the atomic-bomb gamma dose is concerned, whereas some uncertainties still seem to exist for the neutron dose.

DS86 uses a highly sophisticated computer simulation technique for individual dose estimation. In contrast, the key information for this calculation depends totally on the interview information obtained more than 5 years post-bombing, which has never been further validated or refined.

Since the late 1960s, cytogenetic studies have been conducted using peripheral blood lymphocytes. It has been recognized that, on the average, the higher the estimated doses, the higher the aberration frequency. However, exceptional cases do exist. They are called cytogenetic outliers, meaning that their chromosomal aberration frequency is unusually high or low for a particular DS86 estimated dose. Such cases are due either to errors in dose estimation or to differences in individual radiosensitivity. However, our in vitro dose-survival study of lymphocytes using colony formation assay did not provide evidence of marked heterogeneity in radiosensitivity among about 200 individuals as compared to experimental variation associated with repeated lymphocyte tests for a single donor (2, 3). These results support the hypothesis that the existence of outliers is due to errors in dose estimation.

Our recent findings from cytogenetic tests of 2300 survivors in Hiroshima and Nagasaki combined suggest another source of dose error. In this case, the overall dose-response relationship for the frequency of chromosome aberration is about twice as steep in Hiroshima as in Nagasaki. In Hiroshima, the average dose-response relationship did not differ among survivors with different shielding conditions, whereas in Nagasaki, those who were in Japanese houses at the time of the bombing showed a distinctively steeper dose response than those exposed under other conditions. Comparison of survivors who had been in Japanese houses in both cities revealed only small differences (4).

Thus, it has been our wish to test an endpoint other than lymphocyte chromosome aberration versus DS86 dose that might serve as a quantitative indicator of dose. The frequency of mutant erythrocytes lacking expression of one of two alleles at the glycoprophin A locus was once expected to be a useful individual biodosimeter. Unfortunately, individual variation in the mutant cell frequency is so extensive that the assay is not as well suited for individual biodosimetry as it is for use in collective population biodosimetry (5, 6). The only other currently available method is the electron spin resonance (ESR) (or electron paramagnetic resonance) method to measure radiation-induced radicals in tooth enamel.

Enamel covers the crown of a tooth and is unique in the human body because of its substantially inorganic composition and lack of metabo-
lism in contrast to bones. The major constituent is hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ which forms a crystalline structure. About 2.5\% (w/w) of the enamel consists of $\text{CO}_3^{2-}$, which is believed to be incorporated in place of $\text{PO}_4$ to terminate crystal elongation. After irradiation, radiation-induced free electrons can be trapped by $\text{CO}_3^{2-}$ to produce $\text{CO}_3^-$, which is detectable by ESR.

A stable atom carries paired electrons with opposite spin in the same orbit. Atoms or molecules bearing unpaired electrons are unstable and chemically highly reactive, and are thus called radicals. $\text{CO}_3^-$ is a radical species but persists over years if it remains as part of the crystalline structure of enamel. Production of $\text{CO}_3^-$ radicals is linearly proportional to the dose, and hence dose can be estimated by measuring the strength of the ESR signal intensity for $\text{CO}_3^-$ radicals.

When radical-containing materials are located in a magnetic field whose strength continuously changes, resonance of unpaired electrons occurs in response to microwave injection (i.e., absorption of microwaves) at a magnetic field strength characteristic for that particular radical species. Thus, the ESR spectrum is usually represented as magnetic field strength on the X axis and microwave absorption on the Y axis, mostly in the form of a first derivative (7).

**Materials and methods**

Among the more than 300 teeth donated by participants in the RERF Adult Health Study program, 100 teeth from 70 survivors were selected on the basis of the best physical condition and DS86 dose distribution. DS86 dose ranged from 0 to 3.5 Gy.

For enamel isolation, a disc-shaped diamond cutter was used with running water. Before the isolation, the location of each tooth was determined (e.g., upper left third of the mouth) by two or three dentists, followed by careful elimination of tar and tartar. Then, each tooth was cut into two parts consisting of lingual and facial halves. In the present study, only material from the lingual (inner) halves was used.

The isolated enamel was crushed using an agate mortar to sizes of 0.3 to 1.0 mm in diameter for radical measurements by a JEOL Radical Biosensor FR-80 (Tokyo). A field modulation of 100 kHz frequency and 0.32 millitesla (mT) amplitude was used at a microwave power of 16 and 0.4 mW, with a response time of 0.3 seconds and a field sweep of 10 mT in 4 minutes. Each sample was measured 6 times. An internally located manganese (Mn) marker was used at 470 as a standard. All the measurements were performed at room temperature.

To evaluate the total amount of $\text{CO}_3^-$ radicals induced by radiation exposure, it is necessary to subtract the broad background signal, which is believed to be derived from organic materials in enamel. The shape and strength of the background signal vary from one sample to another, and it was difficult to assume a hypothetically representative shape. Consequently, a different approach was chosen to accomplish the subtraction of background noise. As shown in Fig. 1, the background signal intensity is rather refractory to the increase of microwave strength above 0.4 mW, whereas the intensity of the radiation-related signal continues to increase along with microwave power. So we decided to use two different microwave strengths, 16 mW and 0.4 mW, to subtract the background signal from total ESR signal. (Above 16 mW, some samples were found to reveal unusually wavy patterns, difficult to compare with those at 0.4 mW, whereas below 0.4 mW the background signal intensity rapidly shrinks.) Fig. 2 shows an example of such subtraction. It is clear that, even at 0.4 mW, a small fraction of the radiation-related signal still remains in addition to the background signal, and hence the difference between 16 mW and 0.4 mW does not represent 100% of the radiation-related signal. Although this subtraction technique is not yet perfect, we believe that this is the most objective and easiest way to accomplish the subtraction now.

![Fig. 1](image)

The relationship between microwave power and ESR signal intensity

Relation entre la puissance des micro-ondes et l'intensité du signal ESR (résonance paramagnétique électronique)

![Graph](image)

- organic signal for non-irradiated sample – signal organique pour un échantillon non irradié
- $\text{CO}_3^-$ signal intensity for high-dose exposed sample – intensité du signal $\text{CO}_3^-$ pour un échantillon exposé à de fortes doses

Note: The inserts show representative ESR patterns in exposed (above) and non-exposed samples (below). – Les encarts montrent des schémas d'ESR.

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Results

Fig. 3 shows the relationship between ESR signal intensity and chromosome aberration frequency of lymphocytes from the tooth donors. One hundred enamel samples were measured at 16 mW and 0.4 mW for the subtraction. Among these, one failed to provide results because of its unusually wavy pattern at 16 mW. The subtracted signal intensity was first divided by the signal intensity of the Mu marker (internal control). This is due to the fact that the ESR measurement conditions in the cavity become poorer as the total amount of enamel increases in the cavity, which leads to deviation from a linear relationship between enamel weight and ESR signal intensity. The corrected signal intensity was further divided by the total amount of enamel measured for calculation of ESR signal intensity per milligram of enamel.

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Discussion

One exception was found, however, for a man who was exposed at 1.1 km from the hypocentre and whose frequency of aberrant lymphocytes is as high as 35%. His tooth enamel failed to show any sign of radiation exposure, although he had been exposed to the bombing at 15 years of age and reported various acute radiation symptoms. It turned out that the sample was a wisdom tooth, known to develop much later than other permanent teeth, and we concluded that the enamel was most likely underdeveloped at the time of the bombing.

Fig. 4 shows the results of the chromosome aberration frequency plotted against physically estimated DS86 dose. As has been known for many other survivors, good correlation predominates, but some outliers can be seen and will be discussed next.
The chromosome aberration frequencies are unusually low compared with the estimated DS86 doses. — La fréquence de leurs aberrations chromatiques est inhabituellement faible par rapport aux doses DS86 estimées.

Note

In the present study, all the enamel samples were derived from inner lingual halves of teeth to minimize the contribution by dental X-rays, because dental X-rays most frequently come from outside of the mouth while the film is pressed against the gums on the lingual surface of the mouth.

In the near future, we plan to measure all the outer facial halves to determine whether the ESR signal strengths of each half of a tooth coincide. If some samples turned out to give significantly larger signals for facial halves than for lingual ones, it would be wise to exclude such tooth samples from individual dose evaluation. In this way, the contribution of dental X-ray exposure to the estimating of systemic individual dose may be minimized.

**Fig. 4**

Relationship between DS86 estimated dose and chromosome aberration frequency of lymphocytes from the tooth donors

Relation entre la dose estimée DS86 et la fréquence des aberrations chromatiques des lymphocytes des donneurs de dents

Mine the radiosensitivity of each tooth by irradiating enamel samples with known doses of gamma rays. Estimating individual dose would thus require enormous effort.

Fortunately, however, a recent study by Iwasaki et al. (8) demonstrated that the variation among teeth or donors is not so large. After 5 Gy of irradiation, they showed that the coefficient of variation for the mean of the ESR signal was less than 4% for 10 repeated measurements of 4 samples, 5.3% to 11.4% (average 6.1%) for 7 to 26 teeth from 5 cadavers. As a consequence, we feel it pertinent to select samples with a large quantity of enamel and irradiate only half of the each sample with known doses of gamma rays to calculate doses from the ESR signal intensity for all samples.

Another possible problem is contamination of dentin in the enamel samples. Because dentin produces little ESR signal after radiation exposures, the ESR signal intensity per milligram of enamel obviously decreases as the degree of dentin contamination increases. Thus, careful isolation of enamel is critical, otherwise all the samples must be repeatedly measured after *in vitro* irradiation by graded doses.

**Contribution of dental X-ray exposure**

One inherent problem associated with dose evaluation by tooth-enamel ESR is the contribution by dental X-ray exposure. Unfortunately, low-energy photons such as diagnostic X-rays are several times more effective than high-energy gamma rays in producing radicals in tooth enamel (9). Thus, although actual radiation doses by diagnostic X-rays may be negligible, their current contribution to the ESR signal may not be.

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By comparing *Fig. 3* and *Fig. 4*, it appears that the chromosome aberration frequency in lymphocytes correlates better with tooth-enamel ESR signal than with DS86 dose. For example, the two outliers marked by asterisks in *Fig. 4* showed aberration frequencies close to the background level although their estimated DS86 doses were 1.6 Gy and 3.3 Gy, respectively. The interview records showed that they were exposed outdoors without apparent shielding. Interestingly, their ESR data for tooth enamel failed to suggest any high-dose exposures, in agreement with the aberration data. Therefore, for these two survivors, it seems most likely that their memory regarding location at the time of the bombing was inaccurate.

Most of the survivors exposed outdoors were blown about by the blast of the explosion. Frequently it is described in their personal memoirs thus, "I found myself struck against a wall, the sky was dark, and I could not understand what happened for a moment." It is not surprising that their memories may not be correct especially when many houses were crushed by the blast and hence proper landmarks were lost. In addition, the interviews were conducted more than 5 years later. According to the DS86 calculation, air kerma dose decreases by nearly one-half for every 200-m increase in distance from the hypocentre. For those survivors who were in Japanese houses
at the time of the bombings, such uncertainty would be minimal. Taking into account these different circumstances, we feel it reasonable to say that lymphocyte aberration frequency and tooth enamel measurements, both of which are manifestations of physical exposure, are more accurate reflections of true dose than the individual memory of precise location at the time of the bombings.

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Summary
One hundred enamel samples isolated from extracted teeth donated by atomic bomb survivors were subjected to free radical measurement by means of electron paramagnetic resonance (ESR). Results comparing ESR with the chromosome aberration frequency in lymphocytes of the tooth donors, and with the physically estimated DS86 dose suggested that ESR data correlated more closely with chromosome data than with the estimated DS86 doses, probably because DS86 may depend on erroneous memory in some cases.

Résumé
Biodosimétrie: aberrations chromosomiques des lymphocytes et résonance paramagnétique électronique de l’émail dentaires chez les survivants des bombardements atomiques

On a mesuré par résonance paramagnétique électronique (ESR) les radicaux libres dans 100 échantillons d’émail provenant de dents extraites chez les survivants des bombardements atomiques. Les résultats compara­
tant l’ESR avec la fréquence des aberrations chromosomiques des lymphocytes des donneurs de ces dents, et avec la dose DS86 estimée par voie physique, ont donné à penser que les données d’ESR correspondaient plus étroitement aux données chromosomiques qu’aux doses DS86 estimées, probablement à cause du fait que les doses DS86 pouvaient, dans certains cas, dépendre d’une défaillance de mémoire.

References/Références