Cytogenetics for dosimetry in cases of radiation accidents and assessing the safety of irradiated food material

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One of the many areas of research initiated by Swaminathan at the Botany Division of the Indian Agricultural Research Institute, New Delhi was radiation cytogenetics, which involves study of induced chromosomal aberrations. These studies had impact not only on elucidating basic mechanisms involved in the formation of chromosomal aberrations, but also several practical applications related to human health. In this review, we briefly summarize two applications, namely biological dosimetry following radiation accidents and safety of irradiated food material.

Keywords: Cytogenetics, dosimetry, radiation accidents, irradiated food material.

Dosimetry in cases of radiation accidents

IONIZING radiation is an extremely potent agent in inducing chromosomal aberrations in all types of cells both *in vivo* and *in vitro*. The types of aberrations induced depend on the stage of the cell cycle irradiated. Irradiation of cells in G0 and G1 stages will yield chromosome type of aberrations (i.e. involving both the chromatids of a chromosome) whereas in G2 cells chromatid type of aberrations are induced. The frequencies of aberrations increase with the dose. The yield of chromosomal aberrations, especially exchange type of aberrations, such as dicentrics for a given radiation dose is similar both *in vivo* and *in vitro*. This characteristic feature can be used to estimate absorbed radiation dose in victims of radiation accidents. This applied aspect of radiation cytogenetics will be reviewed in this part.

Background to the formation of chromosomal aberrations by ionizing radiation

The target for induction of aberrations is DNA. Ionizing radiation induces several types of damage in DNA, such as, single strand breaks, double strand breaks (DSB), base damage and cross links. Among these, DSB have been shown to be the critical lesion leading to radiation-induced chromosome aberrations[1]. Sparsely ionizing radiations, such as X-rays and gamma rays have low LET (linear energy transfer) values and induce ionizations as well as DNA damage and chromosomal aberrations randomly distributed among the cells. This has been shown to be the case following X- or gamma irradiation and induced aberrations fit a Poisson distribution. With densely ionizing high LET radiations, such as neutrons, alpha particles, the ionization tracks will be non-randomly distributed between cells. This characteristic results also in non-random distribution of chromosome aberrations among the cells and at any observed mean aberration frequency, there will be more cells with multiple aberrations and with zero aberrations than expected from a Poisson distribution[2,3]. Two major types of aberrations are recognized, namely exchange aberrations (interaction between two chromosomes) and deletions. Chromosome exchanges include dicentrics and centric rings (asymmetrical exchanges), as well as translocations (symmetrical exchanges). The dose response curve for induction of exchange aberrations induced by low LET radiations is linear-quadratic, exemplifying contributions of both one and two track events and generally fits the equation:

$$Y = A + aD + bD^2,$$

where Y is the yield of dicentrics, D is the dose, A is the background frequency, a is the linear coefficient and b is the dose-squared coefficient. With chronic exposure (low dose rate) to low LET radiation, the yield of dicentrics is linear. Following high LET radiation, the dose response for induction of dicentrics is predominantly linear.

Human lymphocytes

Human peripheral blood lymphocytes are predominantly in dormant G0 (pre-DNA synthetic) stage. The majority of the circulating lymphocytes are T cells (thymus derived), which can be stimulated to proliferate *in vitro* by a mitogen such as phytohaemagglutinin (PHA). This makes lymphocytes ideal target cells for looking for induced aberrations. There are several types of T lymphocytes with different average life spans.

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Radiation accidents

In radiation accidents, it is essential to estimate the absorbed dose in the victims to help plan their therapy. In most accidents, no physical dosimetry is available. Even in situations in which physical measurement is feasible, an independent estimation by biological methods can be very useful. As blood can be drawn easily in a non-invasive way, the circulating lymphocytes have been employed as target cells for estimating absorbed radiation dose in case of accidents. Since *in vitro* and *in vivo* irradiation of lymphocytes induces similar yields of chromosome damage per unit dose, the absorbed dose can be estimated by comparing the observed frequency of aberrations in the lymphocytes of accident victims to dose response curves generated from *in vitro* experiments[2]. To assess the extent of damage in the lymphocytes, several end points such as dicentrics, translocations, micronuclei and fragments in prematurely condensed chromosomes (PCC) can be used. The choice of the end point depends on the urgency and accuracy needed in the radiation dose estimate. However, the most commonly employed end point is the frequency of dicentrics. In most of the radiation accidents, such as the ones which occurred in Mexico, Chernobyl, Goiania (Brazil), San Salvador, Istanbul (Turkey), etc. dose estimates were made mainly using the data on the frequencies of dicentrics.

One of the authors (A.T.N) had the responsibility to set up the biological dosimetry laboratory for the Brazilian National Commission for Nuclear Energy in Rio de Janeiro in 1986 under the auspices of the International Atomic Energy Agency and therefore, was involved in estimating radiation doses of the victims of Goiania accident. In this accident, which occurred in 1987, a 1375 Ci cesium-137 teletherapy unit was broken by ignorant individuals looking for scrap metals. The radiactive cesium which was in the powder form was distributed to several individuals and the whole neighbourhood was contaminated. Of 11,200 individuals monitored, 249 were contaminated either internally or externally. Immediately after the detection of the accident, more than 110 samples of affected persons were analysed for the frequencies of dicentrics and rings in the lymphocytes[4]. A prerequisite for conducting radiation dosimetric studies is the availability of a reliable dose response curve generated following *in vitro* irradiation of lymphocytes with the same type of radiation involved in the accident, in the participating laboratory. Since no dose response curve for low dose rate ^{137}Cs was available at that time, an existing calibration curve generated for ^{60}Co *g* rays at a dose rate of 0.12 Gy/minute was used for estimates. Of the 110 individuals analysed, 29 had an estimated dose of 0.5 Gy and above (0.5–7.0 Gy). When new calibration curves used similar exposure rates as in the accident, the estimated doses were reduced by about 20% (ref. 5). Though most of the individuals received an inhomogeneous dose, suggested by the presence of localized lesions in the skin, all cases except six showed a Poisson distribution, suggesting whole body irradiation. In this accident, the exposure pattern was complicated (fractionated, protracted and internal) resulting in most cases in a distribution indistinguishable from Poisson. The dose estimates made by this method were very similar to those generated by physical and chemical dosimetry. The individual doses estimated fitted very well with the chronology of events in this accident.

Based on the persistence of lymphocytes carrying dicentric chromosomes following *in vivo* exposure, the mean life time of lymphocytes can been estimated. These estimates made by earlier studies vary from 530 to 1600 days[6]. Some classes of lymphocytes persist for more than 50 years as exemplified by the atom bomb victims from Hiroshima and Nagasaki who still carry circulating lymphocytes with dicentrics. The Goiania accident provided a good opportunity to estimate the average life span of lymphocytes *in vivo*. In the follow up studies of 10 exposed individuals, the disappearance of lymphocytes carrying dicentrics was monitored and a mean half-life of about 130 days with a range from 95 to 220 days was estimated[7]. The lifetime of lymphocytes may vary according to the health status of the exposed individuals. Most of the victims under study had a mild to severe leukopoenia, which is expected to accelerate the repopulation of lymphocyte pool. In the case of two victims, who had high^{137} Cs body burden, the aberration yield increased initially with time (up to about 110 days) in reasonable agreement with estimated doses due to internal contamination and began to fall following decorporation of radioactive Cs.

One can monitor radiation workers in nuclear industry who receive cumulative exposures. as well as workers accidentally exposed to radiation, using the frequencies of dicentrics in lymphocytes[8]. From the distribution of dicentrics among the lymphocytes one can make dose estimates both for whole body or partial body irradiation, as well as for exposure to high LET or low LET radiation or a mixture of these two types of radiation[2].

Retrospective dosimetry

In cases where biological dosimetry could not be performed immediately following a radiation accident, it can be done later. As pointed above, the lymphocytes have limited life span and dicentric carrying lymphocytes will be eliminated with time. Reciprocal translocations are relatively stable and in theory can be used successfully for retrospective dosimetry. Fluorescence *in situ* hybridization (FISH) technique using chromosome-specific DNA libraries allows one to detect translocations with relative ease[9]. Though claims have been made that from the frequencies of translocations detected by FISH, one can estimate radiation doses decades after exposure, based on the study of the atom bomb victims from Japan[10], further studies have shown that there are several limitations in this approach. It is assumed that translocations and dicentrics following irradiation are formed in 1 : 1 ratio and hence the frequencies of translocations retrospectively

detected should reflect the initially induced dicentrics. *In vitro* studies employing FISH have shown that many more translocations than dicentrics are induced for a given dose. In a detailed 8-year follow up study on the victims of Goania radiation accident for which data on initial frequencies of dicentrics are available, the frequencies of translocations have been determined[11]. This study demonstrated that it is feasible to use translocation frequencies to estimate radiation dose retrospectively only in cases where the exposure dose is about 1 Gy and below. At higher doses the frequencies of translocations decrease, similar to dicentrics, though at a slower rate. However, translocation frequencies can be helpful to estimate the cumulative doses in individuals working in the nuclear industry[8]. Populations living in the monazite area in Kerala who are chronically exposed to high LET radiation at a low dose rate will form an ideal cohort for such a study, using FISH technique to detect accumulated translocations. It is a pity that such a study has not been taken up, though the FISH technique has been in use for the past 20 years. Such a study could demonstrate if this population has developed a natural defence mechanism against radiation damage, as there appears to be no increase in the incidence of cancer or childhood mortality in this population.

Fingerprint of past radiation exposure

Several types of translocations are induced by ionizing radiation. Interchanges such as reciprocal, terminal, interstitial and complex translocations are formed between two or more chromosomes, whereas intrachanges formed between the arms of one chromosome (pericentric inversions) or between segments within one arm of a chromosome (paracentric inversions) are formed. It has been observed high LET radiations such as neutrons induce many more complexes and interstitial translocations in Chinese hamster splenocytes[12], which has been confirmed in other studies using human cells. Intrachanges are relatively stable aberrations and remain in circulating lymphocytes over decades after exposure in plutonium workers from former USSR and their high frequencies can be used as a signature for past exposure to high LET radiation[13].

In conclusion, radiation cytogenetics has several practical applications, one of which is biological dosimetry, which has been exploited successfully in many radiation accidents all over the world. This approach is also useful in discriminating between false claims of exposure to radiation and real accidental exposure and is routinely used in national radiation protection agencies.

Assessing the safety of irradiated food material

During the period 1927–1945, the effects of ionizing radiation on living systems were largely interpreted on a biophysical basis. The target or treffer theory of radiation action[14–16] was based on the linear relationship observed between dose and effect. However, the discovery of 'oxygen effect' in radiobiology[17–19] strongly suggested a chemical (physicochemical as well as biochemical) pathway in the actions of ionizing radiation in cells and organisms. The end of the Second World War in 1945 marked a spurt of intensive research on the biological effects of ionizing radiation both for peaceful applications and for setting the radiological protection standards.

It was then that Stone *et al.*[20] reported the production of mutations in *Staphylococcus aureus* by irradiation of the substrate and the possible mutagenic activity of the radiolytic degradation products of the culture medium. Further studies[21] brought out the possible role of peroxide in the adverse biological effects of irradiated broth. Wagner *et al.*[22] showed that both the irradiated medium and peroxide enhanced the mutation rate in *Neurospora*. While these findings of basic value supported a chemical pathway in the development of radiobiological effects, the potential use of ionizing radiation in destroying the food spoilage microorganisms also came to light. One who realized the implications of these findings much in advance and also envisioned its usefulness as early as 1958 is M. S. Swaminathan. In his article, 'Atoms and agriculture' in *The Statesman* (6 October 1958), he wrote, 'The use of ionizing radiation in food preservation is based on its ability to destroy the microorganisms and insects which cause food spoilage. The particular attraction that irradiation offers is that it does not lead to any appreciable rise in temperature of the foodstuff during treatment. The use of radiation thus opens up the possibility of a wider distribution of perishable foods in a fresh state'. At the same time, he emphasized the urgent need for rigorous genetic toxicological evaluation of the safety of the irradiated food materials for human consumption. Keeping in view that bulk of the above-mentioned reports on the *indirect effects* of ionizing radiation (irradiated medium) involved prokaryotic test systems, he organized research to investigate the possible clastogenic activity of irradiated substrate/media on the unirradiated eukaryotic systems such as plant meristematic cells, human peripheral blood lymphocytes, and sex-linked recessive lethal mutations as well as visible mutations in *Drosophila melanogaster*.

The first of a series of papers elucidating the clastogenic activity of indirect effects of radiation was published by Natarajan and Swaminathan[23] who had treated *Triticum monococcum*, *T. dicoccum* and *T. aestivum* and *Allium cepa* bulbs with X-irradiated water and cultured the embryos of *T. monococcum* and *T. dicoccum* in irradiated White's medium. The authors found increased frequency of chromosomal aberrations which they attributed to free radicals/biochemical products resulting from radiolysis of water and the medium. A little later, Natarajan[24] reported that irradiated thymidine solution (0.7% irradiated with a total dose of 7.5×10^6 rad (7.5×10^4 Gy) of gamma rays) caused a high frequency of chromosome aberrations in the root meristems of barley.

During the early 1960s, Swaminathan and his co-workers published a series of significant papers[25–29], which all clearly established the clastogenic activity of irradiated potato mash, fruit juices and culture media. In particular, the observation[26] that the barley embryos cultured on X-irradiated potato substrate (20 kR/0.20 kGy) and stored at 2–3°C for about seven months showed 8-fold increase in the frequency of occurrence of cells with micronuclei was of particular concern. In a critical review, Kesavan and Swaminathan[30] have discussed in detail all aspects of food irradiation from the point of view of their genetic safety for human consumption. The major points in conclusion are:

1. Potatoes irradiated with 6–10 kR (0.06 to 0.10 kGy) do not exhibit any detectable cytotoxic effect. The earlier experiments used much higher doses 20 kR, (0.2 kGy) and the test systems consisted of plant meristems, which do not have detoxification systems as of animals particularly the mammals.
2. In the cytological studies, it was demonstrated[27] that there occurs a decrease in pH and an increase in the peroxide content of the irradiated fruit juices. Bradley *et al.*[31] reported that when the pH of the irradiated sucrose solution was between 4.6 and 7.2 no more chromosome aberration was found than by control solutions, but at pH values lower than 4.6, the percentages of abnormal anaphases in root tips of *Vicia faba* exposed to either control or irradiated sucrose (2%) solutions increased with decreasing pH. However, Kesavan, *et al.*[32] found that the decreased pH of the irradiated sucrose solutions is not the major cause of the observed chromosomal aberrations and growth inhibition. They implicated radiolytic products with considerable life-span. In this regard, the detailed review[33] on the radiation chemistry of carbohydrates is relevant. The radiolytic products of sucrose solutions have further received a great deal of attention[34–38]. These studies have shown that the deleterious compounds produced in the irradiated sucrose solutions appear to be hydroxyalkyl peroxides (HAP) derived from the interaction of radiolytic hydrogen peroxide (H_2O_2) with carbonyl compounds produced in the radiolysis of sucrose. It has also been demonstrated that histidine-peroxide adduct strongly inhibits the growth of *Salmonella typhimurium* in glucose medium at pH 7.0 (ref. 39). The catalase-reactive hydroperoxides predominate in irradiated oxygen-free sucrose, while the catalase-resistant dialkyl peroxides predominate in irradiated oxygenated sucrose solutions[40].

What has emerged finally from the application of cytogenetic methods for the genotoxic evaluation of irradiated food materials by Swaminathan and co-workers is: (i) that milk, fruit juices and many liquids rich in sugars are just not suitable for preservation by irradiation. This is because the radiolytic products of water are the major cause of the degradation of the organic molecules (e.g. sucrose, glucose) as follows:

$$H_2O \rightarrow {}^\circ OH, {}^\circ H, e^-_{aq} \quad (1)$$

$$RH_2 \rightarrow {}^\circ RH + {}^\circ H \quad \text{(Direct effect)} \quad (2)$$

(organic molecule)

$$RH_2 + {}^\circ OH \rightarrow {}^\circ RH + H_2O \quad \text{(Indirect effect)} \quad (3)$$

When O_2 is present, °RH is converted into °RHOO. When water is absent, the *in situ* °RH and °H can undergo harmless recombination:

$$ {}^\circ RH + {}^\circ H \rightarrow RH_2 \quad \text{(Restitution)} \quad (4)$$

The restitution of °RH to RH_2 is indeed favoured by storage at high (~37°C) temperatures even if oxygen diffuses slowly into the irradiated seeds[41].

(ii) The studies by Swaminathan and his co-workers with irradiated potato mash substrate essentially brought out that both the dose of irradiation, and the temperature as well as the duration of storage are important. It is noted that Chopra and Swaminathan[26] had stored irradiated potatoes (20 kR/0.20 kGy) at 2–3°C for about seven months and these potatoes still exhibited clastogenic activity though much reduced. In this regard, the studies by Ehrenberg and his co-workers[42–44] are of relevance. They showed that the main fraction of the free radicals induced in food by irradiation have a half-life around 48 h at 25°C. After six months of storage at +25°C, the radical content was zero or less than one percent of the value, whereas at –20°C, the decay was slower, leaving 10–20 percent of the initial concentration.

It should, therefore, be appreciated that in radiation preservation of potatoes, onions, food grains, spices, etc. the very purpose is to extend their shelf-life at temperatures between 15 ± 3°C (potatoes) and 30 ± 5°C (for grains, pulses, spices, etc.) for at least three months to one year or more. The permitted doses of irradiation are also relatively much lower than used in experimental studies.

The extensive studies by Kesavan and his students[41,45] clearly brought out the influence of initial seed moisture content and post-irradiation hydration temperature on the kinetics of reactivity towards oxygen or decay of oxygen-sensitive sites in barley system. It was also evident that a slow process of 'thermal annealment' of the radiation-induced free radicals results in their harmless decay. In the case of irradiated (75 krad or 7.5 kGy) wheat, the storage at the summer room temperatures (35 ± 5°C) would result in harmless decay of the free radicals. While these irradiated grains are safe from stored grain pests, there will be no development of post-irradiation long-lived/stable radiolytic products. In fact, quite extensive cytogenetic investigations by the Bhabha Atomic Research Centre (BARC), Mumbai, have unequivocally established that even the *freshly* irradiated (75 krad/7.5 kGy) wheat fed to mice and

rats does not result in genotoxic effects and are absolutely safe for human consumption.

A two-member committee (P. C. Kesavan and P. V. Sukhatme) appointed by the Ministry of Health and Family Welfare, Government of India in 1975 carefully examined all the published as well as the unpublished data and concluded in their report that the irradiated (75krad) wheat was safe for human consumption. The details are published elsewhere[46]. The Food and Drug Administration (FDA), USA cited the report of the Kesavan–Sukhatme Committee and published their *final rule* in 1986 that the gamma-irradiated (75 krad) wheat is absolutely safe, and could be consumed by the humans *with impunity*.

Thus, starting with his vision in 1958, that ionizing radiation could be used to destroy the pests and organisms, which cause the spoilage of food, and through a series of cytogenetic studies with his students and co-workers, Swaminathan has played a very significant and responsible role in establishing the food irradiation programme in India on a safe and sound scientific premise. The Bhabha Atomic Research Centre (BARC, Mumbai) with its own in-depth research on genetic toxicological safety of irradiated food materials has come to the *same* conclusion that *liquids rich in sugar* (e.g. milk, fruit juices) are not suitable for radiation preservation, while on the other hand, food grains, spices, pulses, fish, poultry, meat and beef irradiated at the *prescribed* doses *using gamma rays* or electrons up to 10 MeV are absolutely safe. With these radiation sources, there is no induced radioactivity in the food materials. The sprout inhibition doses for potatoes and onions are really in the low dose (0.06 to 1.00 kGy) regime.

It is indeed very gratifying that the Ministry of Health and Family Welfare, Government of India has given clearance for trade in irradiated food materials ranging from potatoes, onions, turmeric, garlic and ginger for sprout-inhibition, mangoes for delaying the ripening; wheat, rice and pulses for disinfestation during storage and fish, poultry, meat, beef and spices for sterilization against pathogenic microorganisms. Food irradiation is now widely accepted in the globalized international trade and it is emerging as an energy-effective, post-harvest processing and preservation technology.

Down the memory lane, we feel very fortunate to have been part of the vibrant school of cytogenetics led by M. S. Swaminathan during the 1950s and 1960s and to have been able, in a small measure, to carry his passion for cytogenetics forward in our own ways for the benefit of science and society.

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