International Workshop on Radiation Health Effects at Low Doses or Low Dose Rates 16-18 Feb 2004

From physical tracks to radiation protection

This three day meeting began with a presentation by Dudley Goodhead, MRC, regarding track structure and the biological implications. Dudley overlaid observed tracks from high and low LET radiation onto biological structures such as cells and DNA to indicate the type and nature of the damage which radiation can cause at the cellular and molecular level. 1mSv per year causes of the order of 10^{-4} double strand breaks per cell per day. For beta/gamma radiation 20% of these are complex, for alpha radiation this rises to 73% Dudley also gave some useful information regarding the background rate of DNA damage which results from processes other than exposure to ionising radiation. In particular he quoted that the background frequency of double strand breaks is of the order 0.1 per cell per day. Although he did point out that these breaks are not usually the complex type caused by exposure to ionising radiation. Dudley briefly discussed the biological response to damage and proposed that different thresholds may exist on the dose-response curve for low and high LET radiation, e.g. no threshold for high LE and, 0.1-0.5Gy for low LET X-rays.

The second presentation, by Albrecht Kellerer, discussed the problems caused by the change from Quality Factor to Radiation Weighting Factor, particularly for neutrons. This has led to a fundamental difference in the way radiation dose is calculated for people and means that the quantity which we call 'dose' can no longer be measured in the laboratory. The reason for the change was to allow effective dose to indicate the risk of harm from the radiation exposure.

In the final presentation of the first session, Herwig Paretzke (whose birthday the meeting was celebrating) discussed the problems faced by those trying to establish appropriate radiation protection arrangements when the actual risk of exposure to ionising radiation is not clearly defined. He stated that ideally we would like to know: the types of radiation health effects; the shapes and magnitudes of the exposure/time/effect relationships (and how these vary with the effect); the locations of sensitive cells and their biological response; human, rather than animal, sensitivity; and be able to identify the markers for causes of different diseases. Herwig also briefly touched on the effect of radiation exposure of the environment. He stated that a recent IAEA report, number 332, found that there were no observable effects on the environment, i.e. plants and animals, at dose rates less than 1mGy per day.

Genome structure and radiation effects

Christoph Cremer began the second session by discussing cellular structure. In particular he described how fluoro in-situ hybridisation (FISH) was used to detect where genes were within cells. This showed that in general the gene-rich parts of the cells were towards the interior, protected by gene-poor regions on the periphery. FISH was also able to show that inactive chromosomes were spatially small than their active counterparts and were therefore less likely to be damaged by radiation. These experiments may have wide ranging consequences, as current models assume that genetic information is distributed homogeneously within cells and this work has shown this not to be true.

The second presentation, by Dietrich Harder, considered the biological effect of very soft X-rays and low energy electrons, including Auger electrons. He has used Monte Carlo calculations to determine the number of high energy depositions from such radiation over biologically significant distances of a few 10's nm. Electrons lose approximately 500eV per interaction, where a number of track ends fall on a single cell, multiple lesions may be formed on the DNA. In addition to these direct effects, the increase in energy in the cell may cause denaturation of chromosomes. This work has found that even though they have very low energy, Auger electrons are highly biologically effective.

Francesca Ballarini gave an interesting presentation regarding the risk of Chronic Myeloid Leukaemia (CML) following exposure to radiation. Again is appeared that clustered DNA damage, leading to complex lesions, was most important. Overall a lifetime risk of 0.004 per Gy for contracting CML was found, with the dose-effect relationship being quadratic below 0.1Gy and linear up to 1Gy. During the questions, Francesca stated that they had not found bystander effects to be important at low doses.

The final presentation of the session, by Andreyan Osipov, described experiments where mice were exposed continuously to low, 610mGy/yr, gamma radiation. Exposures were measured using TLD. The work found that the damage caused by 200-610mGy of chronic radiation was similar to that caused by an acute exposure of 150-200mGy. Analysis of the mice also gave some evidence that double strand breaks induced at low doses are not repaired as quickly as those from higher doses.

Repair and recombination

Markus Löbrich began the third session by considering the repair of radiation damage in human cells. Markus reported that they had found a readily detectable biological marker, a phosphoylated histone, which could be used to infer the number of double strand breaks (DSB) in DNA. This method has allowed the number of DSB resulting from exposures as low a 1mGy to be determined and the repair process observed. The experiments showed that in non-dividing cultures, the number of DSB which remained unrepaired at low doses was higher than at high doses. However, the work also found that when the cells were allowed to divide, the unrepaired cells did not replicate, implying that the system prefers to use undamaged cells to replenish the population than risk repairing a damaged cell. Obviously at higher doses or dose rates, the damage must be repaired to maintain the population. The work found that the background rate of 1 DSB per 20 cells was doubled following a dose of 1mGy and that the rate of DSB formation was linear from 1mGy to 100Gy. The technique has been used on fibroblasts in blood and used to calculate patient exposures from diagnostic radiology procedures. The results agreed well with calculated exposure. In order to use this technique, blood samples from before and after the exposure are required.

The second presenter, Jean-Marie Buerstedde, discussed the overlap in biological mechanisms for DNA repair and immune response and in particular the fact that cloned cells are often immuno-deficient and radiosensitive.

Prakash Hande discussed the effect that telomere equilibrium may have on tumour progression. Telomerase is a chromosome ‘end-cap’ containing specific proteins and simple repetitive non-coding DNA and is thought to protect the coding DNA within the chromosome. Again a link was made between immunodeficiency and radiosensitivity, although telomerase was identified as the cause of the problem. Telomerase dysfunction was found to increase the accumulation of chromosome aberrations and encourage immortal growth, or tumourogenesis. As a result, telomerase status may be a marker for radiosensitivity.

The next presentation, by Kanokporn Noy Rithidech, considered the irradiation of mice and in particular the levels of nuclear factor kappa B (NF-κB) in bone marrow cells. NF-κB is a marker for response to DNA damage. A significant increase in NF-κB was observed 1 hour after exposures of 100-1000Gy, although the level returned to normal after 4 hours. Again a delayed response to repair damage was observed for mice exposed to lower doses, 50-100mGy.

Friederike Eckhardt-Schupp finished the session by describing an on-going study investigating the radiosensitivity of leukaemia patients. The aim of the study is to find some way of identifying radiosensitive patients before radiotherapy treatment is performed so that it can be tailored appropriately. Multiparametric analysis has been performed on in vitro and in vivo blood samples from patients both before and after radiotherapy. Unfortunately, to date, the study has not shown any clear correlation between the experimentally obtained and clinically monitored reactions to irradiation, although there has been some experimental evidence for altered radiosensitivity in derived blood cells from individuals who had severe clinical reaction to irradiation.

Intercellular signalling

James Trosko began the final session of the day describing the multi-stage carcinogenic process, whereby a trigger must induce a stable change in a cell (including stem cells) which is then replicated without change, i.e. the change is not recognised as damage, and this change lead eventually to uncontrolled replication. However, if the change occurs in a stem cell (recently found in many adult organs and tissues), which is already immortal, then the process requires one less step. Alternatively, cancer occurs after two steps: initiation, or irreversible alteration of a single cell; and promotion, the reversible or interruptible clonal expansion of the initiated cell by both mitogenesis and stopping apoptosis. Mitotic suppression also depends on the behaviour of neighbouring cells being altered. The functional capabilities of the initiated cells include: loss of growth control; insensitivity to anti-growth signals; sustained angiogenesis; limitless replicative potential; and being able to avoid apoptosis. James described normal cell functions (senescence, adaptive response, differentiation or die, proliferation, apoptosis) and stated that 95% of cells are quiescent (not active). With regard to the initiation process, changes in genes are normally accompanied by alterations in gap junction intercellular communication (GJIC), although stem cells do not communicate via GJIC. James concluded that if cellular responses or changes in GJIC are not observed at low doses, then it is unlikely that any significant biological effect, leading to a chronic health effect, would occur.

In the next presentation, Georg Bauer described an alternative signalling process using reactive oxygen and nitrogen species (ROS and RNS). He postulated that tumour formation in vivo is dependant on the progression of transformed cells which are resistant against intercellular induction of apoptosis and chemically induced extra-cellular self-destruction. In particular, he has found that tumour cells interfere with ROS mediated signalling. If a method could be found to either enhance the ROS signal so that the tumour cell responded, or alter the tumour cell so that it responded, then this may prove useful in treating cancer.

Ludwig Feinendegen finished off the last day with a presentation about the effects of low doses on tissue homeostatis. He began with a recap of the background rate of damage to cells, whereby the creation of 10^9 reactive oxygen species (ROS) per day gives rise to 10^6 DNA alterations per day of which 0.1 will be a double strand break (DSB). In comparison, 1mGy of X-radiation gives rise to 200 ROS, or 2 DNA alterations, of which 10^{-14} will cause a malignant transformation which will result in the death of the carrier. Ludwig described how ROS was implicated in cell proliferation, growth arrest, apoptotic cell death (controlled, co-operation with neighbours) and necrotic cell death (uncontrolled, cellular material released, damage response). He stated that cell repair only occurred for exposures less than 0.2Gy and that above this level apoptosis was induced. Ludwig also gave some types of adaptive response, and the timescales over which they were initiated: acute repair was immediate; detoxification occurred within hours; DNA repair occurred between hours and days; and the immune response occurred between days and months. Once initiated, adaptive responses may last for many weeks. The findings of this work support a threshold for stochastic effects, and possible hormesis.

Bystander effect and genomic instability

Barry Michael began this session by considering single track effects. Microbeams were used to irradiate single cells, and in some instances particular regions of those cells. Barry reminded us that a single electron through a cell was equivalent to a dose of 1mGy, and a single alpha particle, 500mGy. He stated that although the biological endpoints of targeted and non-targeted (or bystander) effects were similar, the dose-response relationship were different. For targeted effects, the effect was found to be proportional to dose, whereas for the non-target effects, the effect plateaued at low doses. When 1% of the cells were traversed by an alpha particle, delivering a dose of 500mGy, around 30% of the cells responded. 1-2 Gy of X-radiation was required to give the same response. The bystander effects were observed even when the intercellular distance was greater than that normally associated with gap junction communication, i.e. >100µm. Although there was some evidence that these effects were binary, i.e. may happen or not, at energies greater than 1MeV, the bystander effect was always observed. Protective bystander effects were observed to fade over a period of around 3 hours.

The second presentation, by Christian Streffer, described how genomic instability increases after exposure to ionising radiation and considered the consequences for the development of late effects. The study concentrated on a specific gene, HPRT, on the X chromosome. It found that in this gene, the genetic instability rate was doubled by a dose of 1Gy, that direct radiation effects acted to increase the frequency of deletions but decrease the frequency of point mutation, and that delayed radiation effects decreased the frequency of deletions but increased the frequency of point mutations. The actual changes caused by the different radiation effects were the same as those which occurred spontaneously. A study of uranium miners has indicated that the effects induced by radiation persisted for many years. Christian also presented some data which showed that, in general, humans with a genetic predisposition for cancer also show an increased genome instability. This genomic instability could promote the multi-step process of cancer development by increasing the mutation rate in cells in these individuals.

Mark Little describe three models which has been developed for the bystander effect and in particular transmissible genomic instability. The models assume a different number of stages, between 2 and 5, occurs in the process of genomic instability and the SEER (US surveillance epidemiology and end results) study and A-bomb data relating to colon cancer were used to test the models. In each model the cells can be alive and unaffected, alive and affected or dead (or dying). Transfer between these states is allowed and account is taken of the physical location of cells and repopulation. Although the five stage model was found to give the best fit to the data, it was not much superior to the models with a smaller number of stages. Mark also introduced the idea that the cancer type and progression may depend on the type of cell affected, and in particular whether it was a stem cell.

The next presenter, Mark Hill, described the experimental techniques which are available for studying the bystander effect in vitro by both high and low LET radiation. For high LET, a Pu-238 irradiator with a dose rate of 24Gy/min is used. The alpha particles produced have a range of around 20μm in the cell culture. A cold cathode is used to produce ultra-soft X-rays, Al_{κ}, with dose rates ranging from < 1mGy/min to 50Gy/min. At these low energies, no scattering occurs and the only interaction is the photoelectric effect. Cells are grown on 10μm thick CR39 plates in either mono-layers or tissue systems and are observed to move, sometimes as much as one cell diameter per hour. More than one population may be present on a single plate, although not in direct contact they share the same medium, allowing mediated signal transfers to be observed. Various partial shields are available to restrict the exposure of the cells including slits, grids and half discs. To date the research has found that the signal between populations is transferred by the medium but not initiated by it and that the level of instability induced is not dependent on the number of cells irradiation but the dose delivered. Using the grid, for cells irradiated by soft X-rays chromosome aberrations are reduced, this is not seen for high LET irradiation.

In the final presentation of this session, Munira Kadhim, described the results from her study into the genetic instability in haemopoietic stem cells following low dose low LET irradiation. Earlier work found that genetic instability depends on many factors including genetic predisposition and radiation quality. Early results from the study indicated: that genetic instability was only induced following high LET irradiation (0-1Gy) but not following low LET irradiation (3Gy); that there was no dose dependence; non-clonal aberrations occured, but not in every colony; and that the number of aberrations was higher than expected. More recent results from the study have revised the earlier findng that there was no dose dependence, as this has now been observed for low LET irradiation. Additional tests using pulsed fields have been carried out. These indicate that about 1Gy there is no difference in genetic instability induction for high and low pulse frequencies, but that below 1Gy, low pulse frequencies are more effective at inducing genetic instability. Some dependence on the strain of mice cells used has also been observed, suggesting that the apoptotic mechanisms in different cells are different.

Thyroid cancer in the aftermath of the Chernobyl accident

There were three presentations in this session. In the first, Jacov Kenigsberg summarised the data in relation to dose and age dependence. He gave figures showing that for young children, age 0-6 years, the incidence rate was twice that for older children and that girls appear to be five times more likely to express thyroid cancer following exposure than boys. There is some evidence of a threshold for the induction of the disease, at around 50mGy and spontaneous rates of thyroid cancer also appear to increase post-2000. Jan Kaiser gave an insight into the possible sources of ecological bias in the studies of thyroid cancer following Chernobyl which use aggregate data. He discussed some of the possible confounding factors, such as comparing individuals for large towns and very rural areas, differences in detection and reporting of disease in different areas and general health status in different groups. The excess relative risk for the induction of thyroid cancer was given as 1 case per 5000 person years per Sievert. The background risk of the disease was taken as 1 case per 100000 person years. The final speaker, Horst Zitzelsberger, described how FISH had been used to analyse RET rearrangements in post-Chernobyl thyroid cancers. The study found that RET rearranged cells appeared as clusters within tumours, indicating multi-clonal origin, and that the biological features of the cancer were more dependent upon the patient age than the cause of the disease.

Carcinogenesis

The first presentation of this session, given by Suresh Moolgavkar, considered the role of gestational mutations in colo-rectal cancer. A four stage model was described, where the first stage, the initiation of the cell, occurred in-utero. Using a 'best guess' mutation rate of 10^{-6} to 10^{-8} per cell division in utero, the author suggested that 15-20% of colo-rectal cancer could be caused by gestational mutations.

The second presentation, given by Georg Luebeck, continued the discussion about colo-rectal cancer. A predictive model was proposed to explain the observed incidence of this cancer. This model assumed that acute radiation exposure produces a Poisson distribution of mutated cells depending on the dose and the number of cells available. The findings of the study were comparable with those of other studies, such as BEIR V, which gave excess relative risks between 0.5 – 2. However, it was found that although the time of exposure did not affect childhood cancer risk, but that the later the exposure occurred the greater the risk of cancer during adulthood.

Peter Jacob then gave a review of the analysis of four data sets on human lung cancer with the two-step clonal expansion model. In three of the data sets information on smoking was available and in each of them 95% of lung cancers occurred among smokers. The review found that although the initiation rate depends linearly on dose, where non-linear dose response was allowed, there was a downward curvature of the excess risk at low (100mSv – 1Sv) doses.

In the final presentation, Helmut Schollnberger considered the radioprotective mechanisms within a two-stage cancer model. The model accounts for cell birth, initiation and death. For lung target cells, low doses (1-100mGy) were found to induce error free repair of double strand breaks.

Tumour induction after low-LET exposures

Colin Muirhead began this session with a review of the incidence of childhood leukaemia following the Chernobyl accident. In performing the review it was assumed that the risk of leukaemia was greater than for other cancer types, that the risk was greater for children than adults and that there was a short latency period for the expression of the disease. One difficulty with the review was that most studies are based on aggregated mean dose rates by area or with time and that the quality of data available varies with region and over time. Colin was able to make some conclusions: there is little evidence to date for a raised risk of leukaemia in the 0-14 years age group; there is no proof of an increased risk in infants; the paucity of individual data makes it difficult to quantify any risks associated with radiation exposure from Chernobyl.

In the second presentation, Donald Pierce discussed the age-time dependence of radiation-related cancer risks. He explained how radiation exposure may be considered to be cumulative, in that once a cell has been mutated by radiation, that mutation cannot be undone, however he also considered that as an individual gets older, the proportion of cells mutated by the previous radiation exposure becomes small in comparison to mutations resulting from other factors. As a result, the risk from exposure to ionising radiation persists for the individuals lifetime and although the excess relative risk decreases with age, the absolute excess risk increases with age.

The final presentation of the session, given by Laetitia Lacoste-Collin, investigated whether continuous very low dose gamma irradiation modifies the spontaneous course of lymphoma in SJL-mice (maximum 15 month life span). The study to date has shown that although there was an earlier expression of lymphoma in the irradiated group, the lifespan was around 25 days longer in this group compared with the controls.

Lung cancer due to high-LET radiation

Nina Koshurnikova began this session with an examination of lung cancer incidence in the Mayak worker cohort. Very good information is available regarding the health, status and radiation exposure, including plutonium lung burdens, for this cohort. The study showed a linear dependence of lung cancer risk on alpha dose to the lung, in particular the relative risk of lung cancer was 3.7 at 6.8Sv. Unfortunately for the women in the study, plutonium exposures were so high that many died of heavy metal poisoning, adenocarcinomas and peripheral cancers.

Erich Wichmann continued the discussion with a review of lung cancer due to indoor radon. He considered data from miner studies in Europe, America and Asia. He summarised that the data shows an average excess relative risk of 0.12 per $100Bq/m^3$, with a range of 0.04 – 0.56. Radon concentrations in the studies ranged from around $60Bq/m^3$ to more than $1000Bq/m^3$. One of the studies was discussed in more detail in a later presentation by Helene Baysson. This French study used measured radon levels in individuals homes (both upstairs and downstairs) along with a lifestyle questionnaire to ascertain exposure to radon. An excess risk factor of 0.07 per $100Bq/m^3$ was found.

The next presentation, given by Werner Hofmann, considered low dose high LET exposures of bronchial tissue. In this instance, although the overall dose is low, the dose to an individual cell is likely to be high, with a high probability of a single cell receiving multiple hits. In addition, the deposition of hits is likely to be very heterogeneous. Werner also discussed how exposures to radon and plutonium are likely to differ, with radon likely to decay within the lung, possibly deep within, and plutonium more likely to be cleared biologically before it decays.

The final presentation in the session, by George Monchaux, was concerned with lung cancer in rats exposed to radon. Although rats have different cell types to humans, the work found that fatal tumours grow faster and cause more serious health effects than non-fatal cancers. In addition, supporting work by Trosko, has indicated that alpha particles are indirect promoters of cancer, but that they do not themselves initiate cells.

Cancer among uranium miners

Margot Tirmarche summarised the progress of radon epidemiology in the last ten years in Europe. She concluded that the studies, having taken account of tobacco use, showed that a significant increase in the lung cancer risk with cumulative exposure to radon was observed, even at low exposures but that there was no apparent link with dose rate. The excess relative risk was quoted as 5% per working level month (where 1 WLM = $3.5mJh/m^3$ based on a 170h working month and 3.5mJ is the alpha energy deposited by radon and its daughters). Margot raised some concern that it was unclear whether workers were subject to radon gas or uranium dust, which would impact on the biological processes involved in dose assessment.

The second presentation, given by Agnes Rogel, described the use of the statistical method B-splines to analyse the effect of time since exposure on the risk of lung cancer in uranium miners. This gave a risk factor which varied with time since exposure, reaching a maximum of 4% per WLM at ten years post exposure and decreasing to zero in individuals more than 20 years post exposure.

Ladislav Tomasek concluded the session my discussing the late effects of exposure to uranium dust, and in particular the risk of developing leukaemia. This study highlighted the problem of confounding factors as although no significant increase in risk with radon exposure was observed, there was a risk with increasing duration of underground work. However, an increased excess relative risk with exposure to uranium dust was observed, magnitude 7.1 per Sv, with a latent period more than 5 years.

Non-cancer effects of radiation

Sarah Darby presented an interesting paper on the non-cancer effects, and in particular circulatory disease, of ionising radiation. The A-bomb studies have shown an excess relative risk of heart disease of 0.17 per Sv and 0.12 per Sv for stroke, with an upper bound on the threshold dose for the effect at 0.55 Sv. In a study of breast cancer patients, those who received radiotherapy treatment showed an increased incidence of circulatory disease than those that did not, and the risk was increased for those receiving left side radiotherapy (typical cardiac dose of 17Gy compared to 1Gy for right side) . The excess relative risk postulated was 0.16 per Sv. For those over 50 years of age, the excess risk of circulatory disease was found to be equivalent to the risk of cancer. Sarah also considered patients with peptic ulcers. In this case doses to the heart of the order of 2Gy were not uncommon.

The second presentation, by Per Hall, discussed the effect of exposure to ionising radiation in childhood on cognitive function in adulthood. Per was particularly concerned with the effect of ionising radiation on the development of the synapses which grow rapidly during the first three years of life, reduce between the ages of 8-10 years, and remain steady in adulthood. The study was based on a cohort who had received low doses of radiation in the first 18 months of childhood for the treatment of skin hemangiomam, or birth marks, on the face or head. Doses exceeding 250mGy were received by 17% of the population. Intellectual capacity was assessed at the age of 18-19 years prior to military enlistment. A negative dose relationship was found for both frontal, decrease of 53%, and posterior, decrease of 41%, exposure for cognitive tests relating to scholastic skills and logical reasoning, although spatial recognition did not appear to be affected. The decrease in academic ability was also apparent by the fall of children attending high school from 32% in the unexposed group to 17% in the exposed group.