Tissue and data archives from irradiation experiments conducted at Argonne National Laboratory over a period of four decades

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Abstract

Irradiation experiments conducted on dogs and mice at Argonne National Laboratory, IL between 1952 and 1992 led to creation of archives of paraffin embedded tissues accompanied by extensive datasets with gross pathology and histopathology information. Over the past 40 years these data were investigated computationally, using different statistics approaches. Embedded tissues are used to this day as a source of genomic and mitochondrial DNA for quantitative PCR amplification. Data and paraffin block sections are available upon request—interested researchers should visit the websites <http://janus.northwestern.edu/dog_tissues/introduction.php> for dog and <http://janus.northwestern.edu/janus2/index.php> for mouse archive.

Keywords: tissue archive, gamma rays, fission spectrum neutron irradiation, dose rate, relative biological effectiveness

Introduction

Ionizing radiation is a well known risk factor for many human diseases and especially cancer, based on epidemiological studies of cohorts of Hiroshima and Nagasaki atomic bomb survivors (Kellerer 2000, Little 2009). However, this data is limited to acute high dose exposures, while it is known that different qualities of radiation, total dose, dose rate and fractionation pattern lead to different effects of radiation on cells *in vitro* and *in vivo*. The biological effects of radiation are noted at every level of biological organization; furthermore, response of healthy and tumor cells to irradiation have been proposed to involve different repair processes (e.g. Williams et al. 2008). In order to provide experimental data on different types of radiation exposures, many nations have investigated the effects of radiation exposures in animal models. The National Radiobiology Archives (NRA) project in USA concentrated initially on studies of beagle dogs exposed to ionizing radiation conducted at five DOE laboratories. Subsequently, major studies with different strains of mice were done at Oak Ridge, Brookhaven and Argonne National Laboratories; while plutonium inhalation experiments on rats were conducted at Pacific Northwest National Laboratory. In this review, however, we will focus on two large scale studies done at Argonne national Laboratory (ANL) and the data and tissue archives acquired from these studies: Beagle Dogs Tissue Archive and Janus Tissue Archive.

Beagle Dog Experiment was carried out at ANL between 1952 and 1991 and was supported by grants from the Atomic Energy Commission (AEC), now the Department of Energy (DOE). The effects of external irradiation with Cobalt-60 were the main focus of this study. Beagle dog was selected as a small to medium-sized dog breed and numerous different ‘standard’ tissues and lymph nodes and tissues from organs showing different pathologies were embedded in paraffin blocks. These dogs were subjected either to chronic γ irradiation exposure (22 hours/day) or to more intense fractionated exposure using the same reactor.

Besides the dog study, another large scale experiment was conducted on mice at ANL between 1969 and 1992. The strain used was B6CF1 mice, F1 generation from the cross of C57BL/6 females and BALB/c males; again, a set of standard tissues including lung, spleen, liver, kidney, etc. as well as tissues showing different pathological deformations was preserved in paraffin blocks and set up a tissue archive too. A water-cooled, heterogeneous 200kW research reactor, named JANUS, was designed and built at ANL for exclusive use in experimental radiobiology and became the focal point for a range of radiobiological studies gathered under the name of “the JANUS program.” The program included 49,000 mice exposed either to γ-rays or fission spectrum neutrons. The peculiarity of the neutron exposure study was that JANUS reactor had an extremely low level of γ-ray and thermal-neutron contamination and a comparatively homogenous radiation field in the exposure room permitting large numbers of small animals to be irradiated at a single dose level at one time. Furthermore, dose rate was easily controlled by varying the reactor power level. With few exceptions, all neutron irradiations were matched with γ-ray irradiation exposures in order to acquire the data needed to calculate Relative Biological Effectiveness (RBE) values for the two qualities of radiation for diverse somatic and genetic endpoints.

These two studies had contributed to understanding of radiation effects from subcellular to organism level (e.g. Seed et al. 1977), however, with the development of new biomedical paradigms and novel biochemical methods and statistics we may be able to extract more information from these two archives. At this moment, both beagle dog and mouse tissue archives from ANL are housed at the Northwestern University, and maintained by the Woloschak laboratory. Two websites providing individual animal information have been developed and are getting connected with similar sites in USA and Europe. This review article provides a brief summary of the literature on ANL dog and mouse radiation studies and discusses possibilities for further research.

Beagle Dogs Tissue Archive

Data on chronic ingestion of radium by dial painters that became available in 1950s indicated that there is a very long latent period between radionuclide ingestion and tumor development; instigated by this understanding several life-span studies were initiated at ANL with beagle dogs, which have a median life span of about 14 years. Four separate experiments were done, one focusing on the effects of different internal emitters while other three focused on effects of γ radiation exposures. Total number of dogs involved in all of these studies was about 7000.

**Transplacental Strontium-90 in Immature Beagles:** This experiment was extrapolating possible effects on children born to mothers exposed to strontium-90 from fallout from atmospheric nuclear weapons testing. Fifty three beagles dogs at 1 to 9 days prepartum were assigned in three groups injected with different burden of strontium-90 chloride.

**Daily injections of Strontium-90 in Beagles:** To investigate health risks of continuous exposure to strontium-90 from fallout from atmospheric nuclear weapons testing, ninety eight beagle dogs of various ages and both sexes were segregated in eight groups and exposed to different radionuclide burden through multiple subcutaneous injections of strontium-90 chloride.

**Single Injection of Cerium-144 in Young Adult Beagles:** In order to study the long-term effects of soluble Ce-144, forty nine dogs were injected with this radionuclide and then provided with life-time clinical care, including annual physical examination and blood work-ups.

**Single Injection of Cesium-137 in Beagles:** This experiment was done to examine the organs and tissues at risk following the internal deposition of soluble Cs-137 and the influence of age at exposure on these risk patterns. This study showed acute toxicity in old dogs that was attributed to higher radiation doses associated with increased biological retention of cesium with age. Nearly all of the sixty three dogs of three age and divided into eight groups showed significant liver degeneration compared.

In all cases, dogs were allowed to live out their life span and at necropsy a thorough gross examination was conducted to ascertain a preliminary cause of death as well as other pathological complications contributiong or not contributing to the death of the animal. After histopathological examination of tissues suspected for lesions and an extensive review of representative tissues, a “final” cause of death was determined and entered into the database.

**Duration-of-life γ-Irradiation of Young Adult Beagles:** The purpose of this study was to investigate the effects of duration-of-life exposure at different dose rates on survival and cancer inductions in dogs. External cobalt-60 γ-ray exposure was chronic, it continued until the death of the animals. These dogs were placed in two experimental series: “A” from 1968 to 1970, and “B” from 1976 to 1978. Total of 276 beagle dogs segregated in 10 groups were involved in this study. Test dogs were irradiated 22 hours per day, 7 days a week, in a specially constructed facility. Particular attention was given to dosimetry; all factors contributing to the dose rate and total dose were normalized in the irradiation field by migrating dogs through all positions and orientations with respect to the irradiation source. Control dogs were similarly housed in cages and migrated through positions in the control animal room. Hazard models indicated that hematopoietic failure occurring early in life was positively associated with dose and dose rate. The risk of death from causes other than cancer increased later in the life, again showing dependence on dose and dose rate; overall this risk was lower than the cancer risk. Those dogs that survived long enough to die from cancer, developed different types of cancer after time spans depended only on dose.

**Continuous-Exposure γ-Irradiation until Various Total Doses in Young Adult Beagles:** This experiment was set up to investigate the effect of total dose and dose rate in beagles given protracted whole-body cobalt-60 γ rays exposure in order to: (1) provide a basis of comparison for beagles given continuous irradiation, (2) complement research on mice at ANL, and (3) address practical issues in radiation health hazards in man. Total of 257 beagle dogs, mean age 490 days and separated in 14 groups, were used for this study as well as 86 age matched dogs from the colony controls.

**Leukemogenesis: Duration-of-Life γ-Irradiation of Young Adult Beagles:** This study was done to assay the consequences of duration-of-life exposure at low dose rates on leukemogenesis. The endpoint was the bone marrow structure and function and development of aplastic anemia, myelogenous leukemia, or protracted survival.

Results of these studies were published in peer reviewed literature, books as well as numerous ANL reports. Effects of total accumulated doses of 450, 1050, 1500, and 3000 cGy given at rates of 3.8, 7.5, 12.8, and 26.3 cGy/day (22 hours per day, 7 days a week) were also studied. Firstly, acute death related to hematopoietic aplasia was positively associated with the total dose and the rate at which the dose was delivered. Later, once a dog survived the initial hematopoietic effects of irradiation, the risk of death from causes other than cancer was smaller than the death associated with cancer endpoints (Carnes and Fritz 1991). In this analysis, it was not possible to establish relationship between tumor or non-tumor deaths and dose rate, even though the tumor dependent survival depended on a total dose. In a subsequent analysis risk of death from cancer and causes other than cancer was assessed for continuous radiation exposure with doses between 3 and 540 mGy per day (22 hours per day, 7 days a week). Again, it was established that the time of failure ultimately depended only on the accumulated dose. However, different dose rate and accumulated dose had different effects for the cancer and non-cancer causes of death, which could be presented by different slopes in Gompertz parametric probability model plot (Carnes and Fritz 1993).

Separate analyses were done on two sexes of chronically exposed doges. In females exposed to 7.5 cGy per day (22 hours per day, 7 days a week) three distinct subgroups were detected: (a) low radioresistance group with progressive hematopoietic suppression, with terminal aplastic anemia and survival shorter than 400 days; (b) high radioresistance group with strong but aberrant regenerative hematopoiesis, eventually developing myeloproliferative disease; and (c) high radioresistance group with an early phase of regenerative hematopoiesis, which did not develop myeloproliferative disease (Seed et al. 1993). Prior analysis of blood responses in male beagles showed the same three animal groups with respect to aplastic anemia and myeloproliferative disease (Seed et al. 1989).

Results on dog and B6CF1 mouse (see below) mortality caused by chronic cobalt 60 external beam exposures were compiled in a study conducted in 1989. The data were used to make quantitative predictions of comparable age-specific mortality under comparable exposure conditions. Since the predictions for the mouse fell within the confidence intervals based on the data observed for the beagle, it was concluded that the extrapolation of data between species was a valid concept that could be extended to extrapolations of mortality data between animals and humans (Carnes et al. 1989). However, risk assessments for specific types of cancer are not easy to extrapolate between different species; in this respect beagle dogs are considered a better model than mice. For example, in female dogs, the spontaneous incidence of mammary cancer is higher than any other malignancy (Taylor et al. 1976).

Janus Mice Tissue Archive

Increasing understanding that biological effects of high and low liner energy transfer (LET) radiations are very different (spurred on by acceptance of DNA as the hereditary material and the key target of radiation) led to the idea that experiments with animals are the only certain way to evaluate effects of these radiations on people. Beagle dog studies were already in progress at that time, and it became obvious that the numbers of animals needed for comparative studies precluded use of beagles for this purpose. Therefore, animals selected for comparison of neutrons with γ–rays were mice. The JANUS reactor producing fission spectrum quality neutrons was constructed at ANL, while cobalt 60 irradiator was already present on site. The primary program objectives of the mouse studies at ANL were to obtain data for the development of realistic hazard models of chronic radiation morbidity and mortality in order to understand and possibly predict long-term radiation injury in terms of 1) cell injury and recovery; 2) tissue and organ injury, repair and regulation; and 3) the risk assessment statistics of disease and death (Grahn et al. 1995). JANUS program was supported by the Department of Energy (DOE); total number of mice included in JANUS program studies was about 49000; samples and documentation are available for 39000 of animals.

**JM-2 study:** JM-2 was the first and the largest of the JANUS program experiment series. One of its objectives was to test the additive effect of incremental neutron exposures, given in different patterns over a 24-wk period. While five different exposure patterns were used, in each case a total neutron dose of 240cGy was delivered. Exposures ranged from acute high-dose-rate exposure to a fractionated exposure given as low doses with low dose rate three times per week for 24 weeks. A matching set of γ-ray cobalt 60 exposures delivered a total dose of 855 cGy in 24 week period or as 788 cGy single dose. These γ-ray and neutron exposures compared the influence of changes in dose rate, the number of fractions, and the protraction period on the long-term effects on animal health. A matching set of sham-irradiated controls was included. Key endpoints of the study were life shortening and development of cancer and the data were used for the estimation of relative biological effectiveness (RBE) of different qualities of radiation and exposure regimens. In addition to life shortening gross pathology at death was recorded, often accompanied by histopahological analysis. Here, as well as in other experiments a standard set of tissues and tissues affected by neoplastic and non-neoplastic pathological changes were collected and preserved in paraffin.

A small age-dependence study was also included in JM-2. Two single doses of neutrons or γ rays and fractionated 24 week exposures were delivered to mice 100, 200 and 300 days of age.

**JM-3:** This was a single-dose study composed of seven replicates done between 1974 and 1977. A single dose of 240 cGy of neutrons was given to male mice only. A small dose-rate comparison was also included in the last replicate. One group of mice was exposed for the 20 min period (same as in other six replicates) while a second group was exposed for 8 h.

**JM-4:** There were four experiments under the JM-4 rubric. The basic study JM-4K involved the 24 once-weekly exposures, carried out in 10 replications between 1974 and 1977.

A concurrent study JM-4W, used only females and two total dose levels for γ rays and neutrons each. A sacrifice-series study of vascular damage was carried out.

JM-4L1 study involved four of the same total doses used in JM-4K study, but it included only γ-irradiated males, with the protraction period of 23 weeks with the reduced the dose rate by a factor about 150 compared to the JM-4L1 study. Total doses were delivered over a 22-h day, 5 d/wk for the 23 week. No comparable neutron exposures were done. Irradiations were done in four replications between 1980 and 1981.

The second low-dose-rate study, JM-4L2, involved a 60-exposure, once-weekly regime with the same exposure procedure as JM-4L1, extended the protraction period to 59 weeks. Again, only males were used, and five replications were done between 1983 and 1984.

**JM-7:** JM-7 used 60-exposure, once-weekly procedure to extend the protraction period to approximately half of the normal life expectancy. Exposures began at 100 days of age. Only two total doses of γ rays and neutrons were done, matching the doses used in JM-4K and JM-3. To evaluate the age-at-exposure variable, JM-7 also included a single-dose exposure that was done at approximately 520 days of age in order to match the age of mice at the end of the 60 once-weekly exposure series. Two doses each for γ rays and neutrons were used and these matched the doses used in JM-3 and JM-4.

**JM-8:** This was a duration-of-life exposure experiment, only one done with the mice in the JM series with the intent to link the JANUS program to the beagle dog duration-of-life studies and to compare protraction factors between the fractionated (24 and the 60 once-weekly) and chronic exposures. In JM8 series three weekly dose levels of γ rays and neutrons were delivered. The lowest and highest weekly doses were the same as 60 weekly doses used for the JM-7 study, and the middle dose levels were 17.4 and 1.67 cGy/wk for γ rays and neutrons, respectively. This matched JM-4K 24 week exposure experiements.

**JM-9:** JM-9 was done in five replicates between 1977 and 1978, with two neutron exposures of 5 and 10 cGy. The latter was delivered both as a single dose and as 24 once-weekly regimen.

**JM-10:** A laboratory-maintained long-lived field mouse *Peromyscus leucopus* males were used in JM-10 in order to evaluate influence of long life span on coping with the radiation stress. The dose levels were repeats of those in JM-3 and JM-4K experiments, with single exposures to γ rays and neutrons, and two 24 once-weekly exposures to neutrons.

**JM-12:** This was a small study on males testing the effects of the short-term fractionation. Entire dose was delivered in a single, 2, 4 or 6 fractions at 1week intervals.

**JM-13:** This study was designed to evaluate the potential risks associated with the periodic exposure of to neutrons, such as can be expected at pressurized-water nuclear reactor facilities. The lowest total neutron dose was 2 cGy delivered in 60 once-weekly exposures with a dose rate of only 0.00167 cGy/min. This study also included a series of periodic genetic evaluations of males drawn randomly from the control and irradiated groups during the course of the exposures. The paradigm of 60 week of exposure was selected to approximate a working lifetime (30-40 years in US) for persons in the nuclear power plant industry. Establishing RBE for very low doses (less than 10 cGy) and low dose rate neutrons was the key goal of the study, because the then accepted RBE value of 10 for fission neutrons was believed by many to be an underestimate.

**JM-14:** JM-14 study was the last in the JANUS program, with the primary purpose was to evaluate the efficiency of Amifostine (S-2-[3-aminopropylamino] ethylphosphorothioic acid; or WR2721; or Ethyol) or WR151327 (S-2-[3-methylaminopropylamino] propylphosphorothioic acid) as radioprotectors against life shortening and tumorigenesis. The study used single doses of γ rays and neutrons, and the mice were injected intraperitoneally 30 min before irradiation with the radioprotectors or saline.

In the early data analysis of JANUS studies, the endpoint most often extracted from the data was life shortening; it was considered that this end point summarizes, in a single index, the cumulative effect of all injuries experienced by an organism (Carnes and Grahn 1991). Hazard models were constructed based on data from different exposure patterns such as single (acute) exposures, and fractionated 24 or 60 equal once-weekly exposures in a study by Carnes and Grahn in 1991. Data from the same exposure patterns were used to investigate the fit of ten different dose response models; life expectancy post irradiation was the variable in these analyses (Carnes et al. 1989). Life shortening dependence of gamma ray dose was found to be linear and inversely proportional to protraction period. Opposite effect was noted with neutrons where protraction decreased life expectancy (Carnes et al. 1989).

Analyses conducted after completion of the JANUS program departed from life shortening as key variable, and evaluation of risk for particular tissue toxicities, especially tumors became the focus of research. Of mice irradiated at age of 110 days with a single acute dose or doses fractionated over a period of 24 or 60 weeks, 85% died from tumors as a cause of death. These tumors were mostly lymphoreticular (45-60%), vascular (20%), or pulmonary (35-50%), followed by fibrosarcomas, hepatocellular tumors, ovarian tumors, and tumors of the Harderian, adrenal, and pituitary glands. Subsequently, dose response curves were constructed and RBE values calculated for different radiation qualities, doses and dose rates, and for different tumor types. In specific cases calculated RBE values varied between 2 and 50; tumors of epithelial origin showing the highest RBE values (Grahn et al. 1992). It has been evaluated by Kaplan-Meier analyses that the lowest dose at which radiation-induced mortality caused by primary tumors could be detected is approximately 1-2 Gy for gamma rays and 10-15 cGy for neutrons (Carnes et al. 2002). An interesting study, encompassing several different experiments, screened pathologies induced by doses below the threshold associated with tumor caused mortality (Carnes et al. 2002). It was found that such pathologic changes often afflicted multiple organ systems.

One recent study focused on lung cancer in mice from experiments JM2, 3, 4, 7, 9 and 13 (Heidenreich et al. 2006). Lung cancer promotion and initiation, rather than life shortening, were evaluated by database re-analysis. This study found neutron irradiation to have an RBE of 10 for low dose rate acute exposures, however, RBE value decreased with the increase in dose rate or with low dose rate neutron fractionation (down to only 4 in male mice). On the other hand, fractionated γ-ray exposure had RBE between 0.4 and 0.7 in comparison to acute exposures. In this study gender was also found to be associated with risk differences.

Even more recent study using the JM14 data (Paunesku et al. 2008) focused on late tissue toxicities (regardless of dose) rather than on cancer caused mortality, which was a subject of prior JM14 studies (Grdina et al. 1991; Carnes and Grdina 1992). The database information was now employed differently than in the “original” JANUS studies. Dataset containing results of gross pathological examination and histopathological evaluation was available for most mice from the JM14 study, therefore it was possible to evaluate non-neoplastic effects of radiation. This analysis found that Amifostine modulates 67% of the non-tumor toxicities induced by gamma irradiation and 31% of the neutron induced toxicities. Significant gender differences were noticed for tumors and for non-tumor tissue toxicities alike. For example, radioprotective effects against neutron irradiation in females were not statistically significant, while they showed statistical significance for neutrons for males and for both genders for γ-rays (Paunesku et al. 2008).

Data analysis was not the only benefit of JANUS program studies. Several studies conducted by the Woloschak laboratory focused on analysis of paraffin embedded tissues from JANUS archive. A study by Churchill et al. 1994 was the first one in this series, establishing appropriate controls and reaction conditions for the technique used for these analysis; a polymerase chain reaction (PCR) followed by Southern blotting and hybridization. Template for the PCR was the DNA isolated from paraffin embedded tissues. This first study used control lung and lung adenocarcinoma tissues from mice irradiated with 5.69 Gy of cobalt 60 γ-rays (six animals) or 0.6 Gy JANUS neutrons (18 animals); these doses were considered to be equal in terms of RBE. Despite the fact that these doses had similar effect on life shortening, samples from neutron irradiated mice showed much higher incidence of deletions of the tumor suppressor retinoblastoma (Rb) gene (Churchill et al. 1994).

Second of these experiments used embedded lung tissue or lung adenocarcinomas from forty non-irradiated controls and 80 irradiated mice. These mice were exposed to 24 or 60 once-weekly γ-ray or neutron irradiations; genes queried were tumor suppressors Rb and p53 (Zhang and Woloschak 1997). In γ-irradiated animals Rb fragments 3 and 5, the parts of the gene that encode the pocket binding region, were the ones most frequently deleted. Technique used for this analysis included a polymerase chain reaction (PCR) followed by Southern blotting and hybridization. Frequency of deletion was not, however, significantly different from situation in adenocarcinomas from control mice. This study was extended to p53, and exons 1, 4, 5, 6 and 9 were analyzed. Exon deletion frequency for p53 was most prominent in neutron irradiated samples, moreover, deletions most often included the entire gene rather than one or more exons (Zhang and Woloschak 1997).

Lung and lung adenocarcinoma tissues from the same exposure conditions, were selected once more in order to detect K-ras mutations in normal lung tissues and lung adenocarcinomas (Zhang and Woloschak 1998). Technique used to detect point mutations in codon 12 of K-ras was a polymerase chain reaction (PCR) followed by Southern blotting and hybridization as well sequencing of selected samples. This work showed that lung adenocarcinomas from neutron irradiated mice regularly had codon 12 mutations and that neutron irradiation was more effective in causing this type of mutation than γ–ray irradiation (Zhang and Woloschak 1998).

Virtual and Physical Beagle Dog and JANUS Project Mouse Tissue Archives

At present, archived tissues from beagle dogs and mice are housed at Northwestern University (NU) and maintained by the Woloschak laboratory. The move of the tissue blocks from ANL to NU and extraction of database information from Oracle were supported by the DOE. The data is now stored in several different formats including Microsoft Access and is available to the scientific community upon request. Most transparent information about the tissue archives is a searchable web application posted on websites http://janus.northwestern.edu/dog\_tissues/introduction.php for dog and http://janus.northwestern.edu/janus2/index.php for mouse archive.

This web application was designed to make the information on individual animals accessible to the scientific community and provide a convenient way to conduct a virtual search for the animal tissues and place a request for the physical tissues. For example, it is possible to query the database for type of irradiation treatment and specific pathology, observe the sketches of lesions and tumors observed during necropsy and investigate if a certain tissue of interest may be available in a paraffin block. Sections from the mouse and dog tissues are available upon request; several research groups are currently testing these samples by immunohistochemistry and biochemical experimental techniques which require protein or DNA as a starting material.

Quantitative Real Time PCR Approach for Analysis of Archived Tissues

Collection of tissue blocks for the mouse and beagle dog archives lasted for the entire duration of the experiments (from 1952-1992) and even later, because experimental dogs were allowed to live out their full possible life span. Therefore, majority of the archived paraffin blocks are decades old and the material embedded cannot be used for every technique that would be possible with the more recently embedded samples. Nevertheless, new biochemical and molecular biology techniques are emerging daily and the types of experiments that can be conducted with these samples increases as well. Woloschak laboratory has used these tissues for isolation of DNA, PCR amplification and Southern blot analysis (Churchill et al. 1994; Zhang and Woloschak 1997; Zhang and Woloschak 1998). At this moment, however, a real-time PCR approach obviates the need for PCR-Southern blot hybridization. We have decided therefore to employ RT-PCR in order to develop a new approach for DNA analysis of mouse tissue samples. In this analysis we will attempt to study correlation between development of cancer or other pathological complications and mitochondrial “health” of mice. Studies investigating the behavior of mitochondria with age (Barazzoni et al. 2000); under oxidative stress conditions (Shokolenko et al. 2009; Manoli et al 2007) or in cancer (Higuchi 2007) are increasing. It is even possible that the mitochondria may play an active role in regulation of genomic instability (Veatch et al. 2009; Lu et al. 2009; Chen et al. 2009).

Gene sequences that will be amplified belong to pairs of genes that produce proteins which act together in mitochondria. Normal functioning of mitochondria depends on seamless cooperation between mitochondrial proteins encoded by the nucleus and proteins encoded by the mitochondrial genome. Such proteins are, for example, participants of the oxidative phosphorylation system of mitochondria where they are organized in four large protein complexes (Hatefi 1985). For the preliminary test experiments we focused on a pair of proteins from complex I (NADH ubiquinone oxidoreductase). Nucleus encoded gene was NADH-ubiquinone oxidoreductase flavoprotein 1 (NDUFV1) (Ali et al 1993) while its mitochondrially encoded counterpart was NADH dehydrogenase 1 (ND1). In a series of preliminary experiments we found that we can reliably amplify short segments of these two genes and plot the results of amplification as an absolute DNA amplification calibration curve. Replicates of the real time PCR data have shown that relationship between the independent and dependent variables of this PCR reaction is very, as measured by the R squared value above 0.9 for real-time PCR conditions with gene copy numbers above 1 per microliter of PCR reaction.

To test the primers and amplification conditions selected for PCR amplifications, we made a comparison between kidney and heart for the gene ND1. We found that in the freshly isolated DNA from mouse organs the gene copy number for ND1 in heart exceeded that of the kidney by 18 fold. However, in freshly isolated DNA from radiosensitive *wasted* mice the extent of this difference was not well preserved and was only two fold.

In the next set of real time PCR experiments we will introduce additional gene pairs for comparison and will focus on JANUS mouse samples of kidney, heart and spleen from animals exposed to different dose rate exposures.

Conclusions

The irradiation experiments conducted at ANL between 1952-1991 lead to development of two extensive tissue and data archives. Much of statistical data analysis has been done with these data, nevertheless, as our understanding of the salient radiation issues increases, new approaches for data analysis will be used. This trend is already obvious from the changes in endpoints chosen for analysis; from life shortening to induction of specific tumor types to observation of late tissue toxicities. Situation with the archived paraffin embedded tissues is similar in many respects. As new techniques for dealing with paraffin tissues and forensic samples become available, so will increase the ease and the likely spectra of approaches to analyze the paraffin embedded tissues. Large numbers of animal samples in the two archives produced at the ANL are matched by very few worldwide studies; this represents a resource that can be used to avoid or reduce the scope of new animal experiments and to reach a still deeper and fuller understanding of effects of radiation of different qualities, doses and dose rates.

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