3. Research Center for Radiation Safety

Outline of Research Career:
Dr. S. Takahashi graduated from Kyoto University in 1974, and after completing master course, started to work as a research scientist at the Division of Radiation Hazard, NIRS. He was at MRC Radiobiology Unit, UK as a visiting researcher between 1985 and 1986, and at Department of Radiation Oncology, University of Texas Medical Branch at Galveston, Texas as a visiting professor between 1996 and 1997. He is now the Supervisory Director, Research Center for Radiation Safety (from 2002) and the Supervisory Director, Advanced Transcriptome Research Center (from 2003, dual position) in NIRS.
Contact point (Email): sentaro@nirs.go.jp

Objectives:
The Research Center for Radiation Safety was established on April 1, 2001, when the National Institute of Radiological Sciences (NIRS) was reborn as an Independent Administrative Institution (IAI). Although the Center is a new organization, its activities are underlined by the wide spread experiences and resources that former research divisions related to biology and environmental sciences have accumulated. The research fields covered by this Center are wide and ranging from environmental, biological to medical aspects of radiation hazards and safety. Concentrating research resources into research activity related to radiation safety, the Center aims at a safely utilizing radiation, advance in radiation safety sciences, understanding the basic mechanisms of radiation effects on humans and living organisms, and contributing to the related scientific fields. The development of advanced technologies related to this field, such as development of experimental animals and implementation of advanced measurement technology for ionizing radiation, is also an important objective of this Center. Support for regulation authorities, governmental committees, and international organizations is also provided by the Center. In addition to the research activities, training and education of students and young researchers are actively carried out.

Overview:
In this financial year, the Research Center for Radiation Safety performed all its research activities very smoothly, in part, because the staffs were now familiar with the new systems which were implemented for the Independent Administrative Institution in 2001. Although some problems occurred, for instance, shortage of funding, reconstruction of laboratory facilities, and construction of a new building, we have overcome these difficulties. Judging from the number and quality of the presentations at scientific meetings and the research papers and reports, it can be concluded that the researchers were active and much progress were achieved this year. The number of original papers published by Center members reached as many as 120 papers, and many of them were published in international journals with good reputations. Proceedings of international or domestic scientific meetings included more than 40 papers from us and the number of oral presentations was more than 400. Some young researchers received prizes from scientific societies, such as the Japanese Society of Radiation Research and the Japanese Society of Radiochemistry. This year, we especially tried to present our research activities to newspapers and other media. As a result, 33 articles appeared in newspapers, and the name of our Center became known well to the public.

At present, the Research Center for Radiation Safety consists of two project research groups and eight fundamental research groups. The Center also operates two research promotion sections, and the Nakaminato Laboratory for Marine Radioecology. In the first project research, Research on the Health Effects of Low Dose Radiation, animal experiments on the induction of cancer by 10 MeV neutron was completed. The cancer induction ratio and relative
biological effectiveness (RBE) are being analyzed now. As to the modification of cancer risks, this project group focuses on the combined effects of radiation and other environmental agents, the effect of scid mutation on thymic lymphoma induction, and the effect of Atm mutation on leukemia induction. The second project research, Space Radiation Project, obtained many accomplishments in this year. The international comparison of measurements of space radiation (ICCHIBAN Project), the detection of bursts of solar flares, the determination of radiation dose during international flights, and the development of a new neutron detector are topics of this group. Biological study on the effect of space radiation was also carried out using heavy ion accelerator (HIMAC) operated by the Center for Heavy Ion Therapy, NIRS. It was found that a novel type of mutation was induced after the irradiation of heavy ions beams which mimics space radiation.

The fundamental research groups achieved much progress in their own research fields. The eight fundamental research groups were: the Environmental Radiation Protection Research Group, the Environmental and Toxicological Sciences Research Group, the Radon Research Group, the Redox Regulation Research Group, the Radiation Hazards Research Group, the Transcriptome Profiling Group, the Laboratory Animal Development and Research Group, and the Internal Radiation Effects Research Group. The Nakaminato Laboratory for Marine Radioecology also actively carried out the research. All the above obtained many new and significant scientific findings from the environmental, biological, and physical field to the medical field.

According to personnel, the total number of workers was the same as in the last financial year; about 100 and 110 people for permanent staff and part-time assistant staff, respectively. The number of postdoctoral fellows was increased to 50. Since many collaborative research studies were performed this year, mutual exchange of staff members was frequent. About 150 persons worked as a visiting researcher or a trainee in the Center. The leader of the Environmental and Toxicological Research Groups, Dr. Y. Muramatsu, and the group leader of Internal Exposure Research Group, Dr. Y. Oghiso moved to Gakushuin University, and to the Environmental Science Technology Institute, respectively. For international collaboration, the Center sent many researchers to the foreign institutions and international scientific meetings. Especially, it should be noted that 40 scientists were dispatched to the International Congress of Radiation Research (ICRR) held at Brisbane, Australia in 2003.

Office of Biospheric Assessment for Waste Disposal

Shigeo Uchida, Ph.D., Head

The biospheric assessment of radiation dose to human beings related to the releases of long-lived radionuclides from underground nuclear waste disposal sites is very important for the peaceful use of atomic energy.

For this assessment, radioecological transfer models and transfer parameters are needed. It should be noted that environmental conditions, such as climate, vegetation, soil, affect on these parameters. Besides, agricultural products and food customs in Japan are different from those in Europe and North America. Therefore, we need to have our own data in Japan.

In this office, environmental transfer parameters, such as soil-to-crop transfer factors (TFs) and soil-soil solution distribution coefficients (Kds), have been collected throughout Japan. The transfer model for predicting the radionuclide behavior in atmosphere-paddy soil-rice plant systems has been also developed.
3.1. Low Dose Radiation Effects Research Project

Outline of Research Career:
Dr. Shimada got Ph.D. in 1985 from University of Tokyo for a thesis entitled, "Unique characteristics of primordial germ cells to ionizing radiation in Oryzias latipes". As a post-doctoral fellow at Mizuo Biohoronics Project of JST (1985-1987) and a research fellow at Tokyo Metropolitan Institute of Gerontology (1987-1989), he worked on the activation of innate immunity for cancer therapy and the involvement of macrophages in aging of blood vessels, respectively. Since 1989 at National Institute of Radiological Sciences, major work has been focused on radiation carcinogenesis, including molecular and cellular mechanisms of T-cell lymphomagenesis and mammary carcinogenesis. Current research subjects are the dependence of carcinogenic pathways on genetic background and age at exposure, and the molecular alterations associated with ionizing radiation.

Contact point (E-mail): y_shimad@nirs.go.jp

Objectives:
The objective of this research project is to provide basic information on the risk of cancer induction and genetic effects from low-dose ionizing radiation for radiation protection. This project is classified into three subjects: the biological effects of neutrons; cancer risks of low-dose radiation; and the hereditary effects of low-dose radiation. Data not available from epidemiological studies is obtained using animal models. For the neutron study, the final goal is to determine the energy dependence of the carcinogenic effects of neutron for each organ, and to provide insight into the factors influencing RBE. For the cancer risk assessment, we have focuses on the dose-response modifying factor, which influences the effects of low dose radiation, i.e., the co-existence of environmental chemicals and genetic background. In studying the hereditary effect, we have used a mega-sized DNA sequencing method to determine spectrum and the frequency of mutations in offspring after paternal irradiation.

Progress of Research:

Biological effects of neutrons
After the accident at Tokai-mura in 1999, the cancer risks and fateful effects of low doses of neutrons became matters of concern. The main aim of this program is to investigate the biological effects of neutrons, and to make clear the relative biological effectiveness (RBE) for leukemia and RBE for fetuses, thereby assessing the risks of neutrons. Cyclotron 10 MeV neutrons were first used in this program. About 2660 SPF male C3H/HeNrs mice, a strain susceptible to radiation-induced myeloid leukemia, were divided into 13 groups of 150-250 each: one control group; six dose-groups (0.05-2 Gy) for neutrons; and six dose-groups (0.2-4 Gy) for gamma rays. Mice were treated with single whole-body radiation, and maintained over their life spans. Dead or moribund mice were pathologically examined. As of the end of March 2004, about 1,700 mice (67 percent) were autopsied. The incidence of leukemia increased depending upon radiation dose, to about 10 percent in the highest-dose groups. To study the effect of neutrons on fetal central nervous system, pregnant female C57BL/6 mice mated with male C3H/He mice were irradiated with neutrons (6 doses, at 0.05-1 Gy) or gamma-rays (6 doses, at 0.2-4 Gy) on day 13.5 p.c. Fetal brains at 24 hours after irradiation were used for analysis; the numbers of vital cells and apoptotic cells in a definite area of TUNEL-stained brain sections were examined under a microscope. Incidences of apoptotic cells increased with the doses of neutrons and of gamma rays. This study is still under way, to gather additional data. Since further experiments on the effects of 2 MeV...
or more slow neutrons on a whole body are needed, the new building with the SPF animal facility, the electrostatic accelerator for neutrons, and other laboratories, were completed at the end of December 2003. The accelerator is now being conditioned, but we will be able to start some biological experiments in the near future.

Cancer Risk - Combined Effect of Radiation with Chemicals:
We are living in an environment filled with numerous natural and man-made chemicals. Radiation carcinogenesis in humans is believed to be a result of interaction with these factors, and it is clear that the carcinogenic response of radiation could be influenced by these factors. The aim of this study is to determine the mode and mechanism of the combined effects of chemicals with radiation, especially at low- or threshold-dose range. The cancer models used in the current study are the murine T-cell lymphomas (TL) of B6C3F1 mice and the mammary cancer of SD rats. We have demonstrated that the dose-response curve of TLs after treatment of fractionated X-rays (4 times, at 0.2-2.0 Gy) and ethyl-nitrosourea (ENU; 50-400 ppm) was, respectively, sigmoid and linear, both with threshold doses. Molecular analysis has shown that the loss of heterozygosity on chromosome 11 and *Ikaros* mutations are associated with X-ray-induced lymphomas. For combined treatment, the synergistic effect was obvious for high-dose radiation, while effects were marginal for low- and threshold-dose radiation. It was notable that there was still a threshold for X-rays combined with ENU. Female rats were treated either with gamma-rays (0.5-2 Gy), methyl-nitrosourea (MNU; 20-40 mg/kg), or a combination of gamma-rays and MNU. It turned out that the combined treatment induced adenocarcinomas, but not fibroadenomas, more efficiently than gamma-rays or MNU alone. The H-ras mutation was not frequent in radiation-induced carcinomas, while it was characteristic of MNU-induced carcinomas. The mutation status of *Ikaros* and H-ras in TL and mammary tumors induced by combined treatment is currently being examined.

Cancer Risks of Genetically Susceptible Mice:
To clarify the genetic factors involved in radiation-induced carcinogenesis at low doses, we analyzed the dose-response relationship between radiation dose and the induction of thymic lymphomas (TL), using scid mice that had a mutation in the DNA-PKcs gene. Scid mice were highly susceptible to the development of spontaneous and radiation-induced TL, as compared with wild-type mice: at 0.25 Gy of gamma-rays, TL were significantly induced in scid mice, while there was no induction of TL in wild-type mice. This indicates that a defect in DNA-PKcs is responsible for susceptibility to the development of spontaneous and radiation-induced TL at a low dose, and suggests that the nonhomologous end-joining repair is involved in the suppression of radiation-induced TL. To analyze the pathways involved in the development of TL, we examined the sequence abnormalities of the breakpoints of rearrangements of Notch1 in TL of wild-type and scid mice. Notch1 has been identified as a major oncogene responsible for TL induction. There were at least two pathways for the induction of Notch1 rearrangements: one is the illegitimate V(D)J recombination pathway that operates at cryptic recombination-signal sequences in the Notch1 locus, while the other is micro-homology mediated nonhomologous end-joining (MNHEJ), in which radiation-induced double strand breaks might be involved, and the processed end paired with another end at the micro-homology sequence, resulting in deletions. In the presence of the DNA-PKcs gene, the illegitimate V(D)J recombination functions as a major pathway for generating the deletion of Notch1, while in the absence of the DNA-PKcs gene the MNHEJ pathway acts as a major pathway. Because the illegitimate V(D)J recombination pathway occurs spontaneously, the MNHEJ pathway might be responsible for the significant induction of TL at a low dose in scid mice.

Hereditary Effects of Low-dose Radiation:
To investigate the hereditary effects of ionizing radiation, mutational events and their frequency in mouse germ cells were analyzed by detecting changes in nucleotide sequences (>10^7 bp/dose) at a specific genomic loci and 150 STS in mouse offspring. Male mice irradiated with or without gamma-rays at 1-3Gy were mated with intact females 3 weeks later. This procedure could determine the genetic effects of radiation at the spermatid stage. DNA (5x10^6 bp, in total) from the offspring was analyzed. The mutation frequency of offspring from the male mice exposed to 3Gy gamma-rays was 1.4x10^-7/bp, while spontaneous mutation was not detected (<1x10^-7). Mutation was not detected in offspring derived from male mice irradiated with 1Gy of gamma-rays. These results suggest that the dose response for mutation induction is unlikely to be linear. One new mutation was also detected in offspring derived from male spermatogonia exposed to 3Gy of gamma-rays. Mutation frequency was calculated to be 2x10^-7 /bp/Gy, which corresponds to one-eighth of the
mutation frequency observed in spermatid. Another mutation was detected from offspring derived from spermatid exposed to 0.5 Gy of neutrons. The mutation frequency was calculated to be 2x10⁻⁷, and the RBE of neutron was estimated 5-6. Similar nucleotide sequence analysis of offspring derived from mouse spermatid exposed to 3 Gy of X-rays was performed at the adenine phosphoribosyl transferase gene locus (3088 bp/locus, 537 mice), but no new mutation was detected. The dynamic mutation at the hyper-variable M6-hm tandem repeat was also analyzed in offspring derived from spermatid exposed to 1-3 Gy of X-rays; dynamic mutation was observed in 5-20 percent of these, according to irradiation dose.

**Radiation Effects on Germ Cells**

We have carried out mutation experiments in somatic cells and male germ cells from transgenic mice, after different doses (0, 1, 2.5, or 5 Gy) of ionizing radiation. The transgenic mice used for this were the gpt-delta strain, which carries 80 copies of the bacterial gpt gene per cell as targets for mutagenesis. Results are as follows:

- **Mutation frequencies in somatic cells:** The spontaneous gpt gene mutation frequencies in whole embryos and the spleens of adult mice were 1.1 x 10⁻⁶ and 1.2 x 10⁻⁶, respectively. The mutation frequencies after exposure to 5 Gy of X-rays in whole embryos and adult spleens are 3.5 x 10⁻⁶ and 2.9 x 10⁻⁶, respectively. When the mice were irradiated with 5 Gy of X-rays, the mutation frequencies in somatic cells increased about threefold over background.

- **Mutation frequencies in male germ cells:** Sperm cells were extracted 65-83 days after irradiation at various doses of X-rays, corresponding to the spermatogonia stage at the time of treatment. The spontaneous mutation frequency in male germ cells was 0.4 x 10⁻⁶. The mutation frequency in male germ cells irradiated with 5 Gy of X-rays at the spermatogonia stage was 0.9 x 10⁻⁶.

**Major publications**

1) Yasushi Ohmachi, Yuka Ishida, Takeshi Hiraoka, Tsuyoshi Hamano, Shinji Fushiki, Toshiaki Ogui: Postnatal changes in mice exposed in utero to fast neutrons. *Journal of Toxicologic Pathology*, 17, 63-68, 2004


3) Kyoko Yasumura,* Isamu Sugimura,* Kazuei Igarashi,* Shizuko Kakinuma, Mayumi Nishimura, Masahiro Doi, Yoshiya Shimada: Altered expression of Tif and Dap3 in Ikaros-defective T cell lymphomas induced by X-irradiation in B6C3F1 mice. *British Journal of Haematology*, 124, 179-185, 2004


5) Hideo Tsuji, Hiroko Ishii, Hideki Ukai, Takenori Katsube, Toshiaki Ogui: Radiation-induced deletions in the 5' end region of Notch1 lead to the formation of truncated proteins and are involved in the development of mouse thymic lymphomas. *Carcinogenesis*, 24, 1257-1268, 2003
3.2. Project: "Physical and Biological Protection of Man from Space Radiation"

Kazunobu Fujitaka, Ph.D.
Director, International Space Radiation Laboratory
Contact point(E-mail): fujitaka@nirs.go.jp

Outline of Research:
Dr. Fujitaka and his group continued discussions with an airline company on future collaborations in measurements of cosmic rays, in which Y.Uchihori was actively engaged. Also M.Takada paid a great effort to complete his Phoswitch detector to measure highly energetic neutrons, which could hold a large portion of cosmic radiation. H.Yamaguchi has supported these researches. In addition, N.Yasuda joined physics research group, where he was to count etch tracks on films that were on board a Russian satellite. H.Yasuda continued working as a member of planning section, but worked hours for the group and gave us useful information. And S.Kinbara calculated motions of highly energetic particles. Based on many low-level irradiation experiments, M.Suzuki showed that the mutation rate in human cells, which had been exposed to very low levels of high LET radiation, would depend on time-length after the pre-irradiation. It was one of the most useful information related to the radiation protection in the space. K.Nojima was interested in stress, which astronauts would meet, and had let mice swim in a water pool ("water maze" experiments), and examined whether they could or could not reach the target within a given time. They were previously irradiated by very low radiations. The mice could be good animal model for middle-aged men working in space. Significant results were expected. R.Okayasu was a competent leader in promoting and encouraging these works. He was engaging in his own cellular and molecular studies related to space environment. S.Fukuda and H.Iida have found that the rats experienced physical training showed less bone mineral loss, which could be applied to astronauts.

Objectives:
To find out the most effective detectors, both active and passive, ICCHIBAN project which compares detectors has been done. Also efforts were directed to avoid excessive exposure on air flights, based on estimated doses to any cities of the world. A Phoswich neutron detector was identified as the best for use in various fields. A by-product of this research was the development of automated imaging optical microscopy.

Long term analyses of exposure to high-LET particles can only be done under very limited conditions. HIMAC is basically a machine for medical purposes, which is free only during nighttime. Therefore, to repeat long time experiments, we have to overcome lots of difficulties. Every night (about 8h), we have to place an incubator with carbon dioxide at a point 45 degrees from the principal path of the beam. This gives an exposure level of about 1mGy/h, which is understood as space-like radiation. Biological project objectives lie in research on cell or cellular mutations by exposure to radiation. More attention is being directed to the best use of the micro beam facility. Another important issue in this field is how much the animal brain is damaged after exposure of samples with very weak (about 1mGy/h) irradiation.

To solve another important issue, clarifying the alterations of calcium metabolism, a study of synergetic effects of radiation and simulated microgravity in rats has been done. This lead to a method to reduce radiation-induced damages, and to an examination of the beneficial effects.
Progress of Research:

1) Dosimetry of dose in space.

The ICHIBAN project included many participants from abroad this year. This project will be continued in the future until all data are to converge. The experiments in space were implemented this year, and negotiations with the Russian participants were undertaken. Basic data on heavy ion response of TLDs and CR39 have been accumulated. A prototype of a compact Si detector was put into practical use, and development of the neutron Phoswich detector has been almost completed, though the power supply is still under review. Efforts to make a diamond detector to measure cosmic radiation is in progress. Material selection was based on its excellence in fundamental functions as well as ability to be used in the severe environment of space. For automated imaging microscopy, four patents have been filed in the U.S. and Europe regarding high speed image acquisition. Until recently, we have had neither rockets nor space vehicles. But Russia has very kindly given us an opportunity to use their vehicles. Then, under the auspices of NIRS, Russia, Austria and U.S. have joined together in the experiments. With the accelerator, we have irradiated some known amount of dose, and made data comparisons. Results are finely concentrated around the given dose with reasonable deviations.

2) Dose which we receive on board airplanes.

Measurements of air flight doses obtained by compact monitors (e.g. silicon detectors) and model Monte Carlo calculations, primarily those from Japan, can sustain practical curiosity. With regard to studies of radiation exposure in aircraft, negotiations with an air carrier are continuing. Data were accumulated largely on "route doses", mainly on flights to the US and Europe, and also model calculations by CARI-6 were repeated there. The results have shown that the annual dose received in flights seem unlikely to exceed 6 mSv.

A large solar flare confirmed existence of the Forbush decrease, which started in late October and continued until two weeks later. In this case, the occurrence of a geomagnetic disturbance resulted in decrease of the dose in an airplane. In this case, the magnetospheric boundary was suppressed inward, which refracted low energy components of cosmic rays, as predicted.

3) Cellular and in vivo effects.

We have found the LET dependence on the brain function in mice irradiated with heavy ions at HIMAC and at NSRL (National Space Radiation Laboratory; Brookhaven, NY; as collaboration). As to water maze experiments to examine brain memory, very low level irradiation down to 0.5 Gy was used to simulate space. With carbon ion irradiation, the brain function was recovered to its normal level 20 weeks after irradiation, while with high LET iron ion irradiation, the function was not recovered even at 42 weeks post-irradiation. We also found LET dependence on the blood cells from mice irradiated with various heavy ion particles. Silicon ions (LET = 55 keV/μm) seem to give the strongest biological effect so far. A rat model which shows susceptibility to renal cancer (Eker rat) was used to study the carcinogenic process associated with high and low LET radiation. We found high LET iron ions gave 1.6 times higher incidence of kidney cancer than the incidence with X-rays.

We have been studying the genomic instability phenomenon in normal human fibroblasts exposed to low doses of ionizing radiation. This year we irradiated cells with low-density carbon ions using faint beams in the HIMAC. The beam is about 1 mGy/h, which is like space radiation. The genomic instability was examined by measuring cell killing and mutation induction in cells pretreated with low density carbon ions followed by irradiation with challenging doses of X-rays. The results showed that there was no enhanced effect on cell killing in low-density pretreated samples when compared to untreated cell populations. On the other hand, the frequency of mutation induction, which was measured as the induction of a 6-thioguanine resistant clone focused on hprt locus, of the low-density pretreated cells was much higher than that of untreated cells. These results suggest that the genomic instability was induced in the form of gene mutation by the pretreatment at a low level of about 1 mGy/h carbon ions. This result is interesting from the viewpoint of future space trips.

We have also been studying the effect of high LET radiation using normal and radiosensitive DNA double strand break (DSB) repair deficient cells. The DSB repair deficient cells showed an alteration in cell growth even after ~10 mGy of high LET background radiation. The cell survival with high LET radiation was similar to that with X-rays in the DSB defective cells. This made a great contrast to normal cells which showed a significantly reduced cell survival with high LET radiation when compared to X-rays. By examining the DSB repair process in these cells, we found that one form of DSB repair process called non homologous end joining (NHEJ) was severely compromised in cells irradiated with high LET.
radiation. These results are also interesting from the viewpoint of aging which is related to long term space stays.

4) Effects on bone and its mineral components.
Also done was the effect of bone mass change due to irradiation associated with microgravity. The microgravity environment is realized by rotating an ingenious machine, a clinostat, by which the gravity is diverged for a long time. We observed the bone was rather strengthened when only radiation was left imposed. This phenomenon will be reviewed in the coming period.

Changes of bone mineral density after immobilization and irradiation, 0.5-2.0Gy, were observed within 2 weeks. As 0.5Gy is the dose which humans would experience in a round trip to Mars, it is very important. The shortening of life span has been observed for rats which were exposed to 0.75-1.0Gy at age of 12 months. Observations are still continuing.

Comparisons were made between the radiation exposure alone group and the group combined with radiation and simulated-microgravity induced by immobilization of the hind limb using neuroectomy. The results showed that alternations in bones were determined at an early stage after treatments. Alendronate, which is used as a drug to prevent bone mineral loss in astronauts, has been found to have an optimum effect when the administration starts just after the irradiation. Experiments to examine radiation-induced cancer, shortened life, and bone damage by using young rats should be continued, and we have started to search for a model to examine individual differences in bone mass and genetic factors, and the effects of nutrient constituents in controlled diet supplementation.

Major publications:

Fig.1.
Cross-sectional area of tibial proximal epiphysis of rats increased in the radiation combined with immobilization group (r=0.967) than in the radiation exposure alone group 22 weeks after heavy ion particle whole body irradiation.
3.3. Establishment of Radiation Protection System against Radioactive Materials Released into the Environment

Outline of Research Career:
Dr. Ishigure received a Ph.D. from Nagoya University in 1979 for his study on energy loss of low-energy (keV) electrons in solids. He has had 25 years of experience in research and development on internal dosimetry at NIRS. Between 1985 and 1986 he was at the Medical Research Council, UK as a visiting scientist, where he studied microscopic distribution of enriched uranium within lungs using solid state nuclear track detectors. He has participated in the ICRP Committee 2 task group on internal dosimetry (INDOS) since 2001.

Contact point (e-mail): ishigure@nirs.go.jp

Objectives:
The over 50 nuclear power plants operating in Japan provide one-third of the country's total electricity supply. To establish a nuclear fuel cycle, which is a fundamental energy policy of the government, a commercial-based uranium enrichment plant is operating at Rokkasho-mura and a huge reprocessing plant is under construction. Nowadays, radionuclides are used extensively in the fields of science, engineering, agriculture and medicine. There is a potential risk for radiation exposure with these applications. Therefore, exposures should be controlled so that the doses and risks to individuals do not exceed levels acceptable for the human population. The objective of this research is to obtain scientific information needed to protect the human body from radiation and radioactive materials released into the environment from nuclear and radiation facilities, by clarifying the amount and behavior of radioactive materials in the environment, the intake of the materials by the human body, their behavior within the human body, the doses to the human body and low-dose risk assessment by epidemiologic studies. In particular, this research group conducts marine studies at the Nakaminato Laboratory for Marine Radiocology. Furthermore, half of the research group members serve at the Research Center for Radiation Emergency Medicine, as they are responsible for studies on dose assessment of exposed patients as well as ongoing practical activities in an emergency.

Progress of Research:
1) Behavior of radionuclides around the living environment
(K. Shiraishi, S. K. Sahoo and S. Kimura)
Using ICP-MS and TIMS, ultra-trace analysis is being conducted to develop a new method for specifying radioactive sources and to study relationships between trace elements and human health.

Precise uranium isotopic composition was determined for soil samples collected in the Chernobyl areas and special areas of Japan. The isotope $^{238}\text{U}$ was detected only in the Chernobyl samples. Ratios of $^{234}\text{U}/^{238}\text{U}$ and $^{235}\text{U}/^{238}\text{U}$ were also higher compared with natural abundance. Variation of the ratios could be used to detect the source origin. Whole diet samples are being collected to clarify the relationship between trace element intakes and diseases including cancer in the Chernobyl areas by duplicate portion studies. Approximately 20 radioactive and non-radioactive elements such as U, Th, I, Cs and Sr, have been analyzed for 100 samples. More samples must be analyzed to confirm the results. A dose estimation method by imaging plates was studied to develop a semi-quantitative analysis of radionuclides in contaminated areas. Several kinds of plants collected from the Chernobyl and JCO accident areas were used. It has been found that the sensitivity of photo-stimulated luminescence (PSL) depended on the content of fluorine. PSL had a significant energy-dependent sensitivity at low energy (60keV).

2) Behavior of radionuclides within the body
(Y. Nishimura, Y. Watanabe, S. Homma-Takeda and M. Yukawa)
Tritium dynamics in the reproductive organs and salivary glands were examined in Wistar adult male rats exposed to 3H-thymidine. While the uptake and retention of tritium in the testis and epididymis were similar to those of the liver and kidney, the amounts of residual tritium were high in the prostate and salivary glands, indicating that these organs may act as a radiation source after exposure to tritium.

The effect of long-term administration of a chitosan diet was studied in F-344 female rats. One group was fed a diet containing 5% w/w of chitosan while another group was fed a standard diet, and their survival rates were observed. The average life span was 867 ± 16.8 days in the standard diet group and 904 ± 22.8 days in the chitosan diet group. Thus life expectancy was extended in the chitosan diet group.

The effects of X-ray irradiation on cultured cells of Japanese cedar were investigated. Cell death in the cultured cells was increased dramatically by X-ray irradiation at 5 Gy, which is the minimum dose inducing radiosensitive programmed cell death (apoptosis) in mammalian cells. This was accompanied by nuclear DNA fragmentation, which is typically observed both in apoptosis of mammalian cells and in hypersensitive programmed cell death observed in plant cells exposed to various environmental stresses.

3) Internal dosimetry for radiological protection

(N. Ishigure, T. Nakano, M. Matsumoto and H. Enomoto)

Recently developed biokinetic models of ICRP permit realistic description of the behaviour of radionuclides in the human body. This, however, has made the interpretation of bioassay data extremely difficult. Thus computer programs for implementing these models are in great demand, but very few are available. In the present work the personal computer-based software, MONDIAL2 (monitoring to dose calculation ver. 2) has been developed, that enables users to estimate intake activity and the resulting effective doses from bioassay measurements for both workers and members of the public. This software runs on Microsoft Windows 95, 98, Millennium edition, 2000 or XP and it is distributed by NIRS free of charge.

To harmonize methodology for internal dosimetry throughout Japan intercomparison/intercalibration exercises among facilities are planned. Four sets of seamless BOMAB (bottle manikin absorption) Phantoms were constructed to use for intercalibration of whole body counters. Each set consisted of ten bottles made of polyethylene plastic. One set of them will be used for background counting by filling each bottle with water or KCl solution through a screw-type fill port. The other three sets will be filled with radionuclide in agar matrix. The radionuclides to be used are 60Co, 137Cs and 133Ba.

4) Radiation epidemiology and risk assessment

(Y. Yoshimoto, S. Yoshinaga and T. Tsukagoshi)

We have continued epidemiologic research for health effects of low-dose and/or low-dose rate exposure of radiation and potential radiation risk of the public near a nuclear power plant (NPP) in Japan. Generally it is not easy to quantify cancer risk of medical radiological work. The mortality follow-up of Japanese radiological technologists showed a larger healthy worker effect in non-tumor diseases than in cancers and suggested association of cancers of lymphatic and haematopoietic tissue with radiation exposures in early periods. Superficial increase by ecological studies can raise a social concern even for small radiation risks due to NPP routine operations. Our recent analysis showed no increased risk for solid cancers or cancers of digestive organs in areas with a Japanese NPP. Excess risk of thyroid cancer has still been seen in the former Soviet Union following the Chernobyl accident. These findings were summarized as a publication of cancer risk assessment for low-level exposure including non-radiation occupational/environmental circumstances. Cooperation with other research institutes in Japan or abroad has continued for studying health effects of occupational exposures. Besides, we took up effects of modification of radiation-induced cancers and exposures from radon in drinking water, We have accepted a Sri Lankan researcher as an exchange scientist.

5) Distribution of radionuclides in the ocean


We reported an analytical method for 239Pu and 240Pu in marine sediment samples which uses quadrupole ICP-MS. To avoid the interference of uranium hydride in the determination of 239Pu, a simple anion-exchange chromatography system was employed for the separation and purification of Pu from the sample matrix. A sufficient decontamination factor of 1.4×10^5 for U was achieved. High sensitivity for Pu determination was obtained, which led to an extremely low concentration detection limit of ca. 8 fg/ml (0.019 mBq/ml for 239Pu; 0.071 mBq/ml for 240Pu) in a
sample solution or an absolute detection limit of 42 fg in 5 ml sample solution by using the shield torch system under normal plasma conditions. The method was validated by the analysis of $^{239,240}$Pu and $^{242}$Pu/$^{239}$Pu ratio in IAEA 368 (ocean sediment) reference material, the analytical results indicated that the accuracy of the method was satisfactory. The developed method was successfully applied to a study on Pu behavior in the sediments from Sagami Bay, Japan. The observed high $^{240}$Pu/$^{239}$Pu ratio in the sediment core indicated that there was additional Pu input derived from Bikini close-in fallout in addition to the global stratospheric fallout.

6) Mechanism of accumulation of radioisotopes by marine organisms

(T. Ishii, M. Nakahara, M. Matsuba, H. Kaeriyama and K. Oginuma)

We are studying about various factors controlling the bioaccumulation of radioisotopes. Information on feeding rate of fish in various environments is important for not only understanding fish ecology but also for getting basic parameters to rear fish under experimental conditions allowing evaluation of physiological characteristics including excretion rate of radionuclides. Traditional analysis of feeding rate of fish in actual environments includes counts, frequency of occurrence, volume or weight of individual prey items and gastric evacuation. These methods are time consuming and the estimates cannot be used for generalization if food consumption varies between days or seasons. Daily feeding rate of the bastard halibut Paralichthys olivaceus taken off the Pacific coast of Aomori Prefecture was estimated by a radioisotope method. The feeding rates were obtained by dividing the daily intake of $^{137}$Cs by the concentration of $^{137}$Cs in the food. The concentrations of $^{137}$Cs of the bastard halibut and the stomach contents were measured and using these data together with previous information on the absorption and the retention of the $^{137}$Cs in this species, the daily feeding rates were estimated. The radioisotope method gave the mean daily feeding rate of 3.7±0.4% of the body weight for the bastard halibut during the period of October to December.

7) Assessment of impacts of radioactive substances released into the marine environment

(T. Watabe, S. Yokosuka, A. Kurosawa)

Marine organisms sometimes show a high affinity specifically to a chemical element or a radionuclide and accumulate it to levels high enough to be readily detected by ordinary measurement techniques. Such organisms have been often used as a "biological monitor" in an environmental surveillance program not only for just monitoring the releases of radionuclides but also for tracing the temporal and spatial changes of their distribution in the marine environment, since the level of radioactivity released under control is generally too low to be detected directly in seawater. In the present study, radioactivity measurements were carried out for marine organisms such as a common species of brown algae (Sargassum thunbergii) and mollusks including some species of gastropods of the family of Buccinum and squids (Todarodes pacificus, Thyasoreuthis rhombus, etc.) for exploring the marine environment background levels of $^{90}$Tc (half-life: 2.111 × 10$^5$ y) and $^{108}$Ag (half-life: 418.21 y). The geographically wide distribution of the organisms made it possible to compare the background levels of radioactivity between coasts in Japan. In addition, the comparison of the specific activity of $^{108}$Ag in the viscera of the mollusks among the species inhabiting layers at different depths allowed a general trend of vertical distribution for the nuclide to be drawn.

Major publications:


3.4. Environmental and Toxicological Sciences Research Group

Outline of Research Career:
Education: 1983, Yokohama National University (BE in safety engineering); 1985, Tokyo Institute of Technology (ME in environmental chemistry); 1989, Tokyo Institute of Technology (PhD in environmental chemistry)
Professional Activities: 1989-present, National Institute of Radiological Sciences
Research Interests: Environmental chemistry, geochemistry, and radioecology
  * Multi-element analyses of environmental samples (such as soil, plants, mushrooms, and earthworms) by ICP-MS and ICP-AES, with special emphasis on the chemical form of the elements.
  * Behavior of radionuclides and related stable elements in ecosystems, with special emphasis on the role of biological activities.
Contact Point (E-mail): s_yoshida@nirs.go.jp

Objectives:
The recent rapid progress in technology and industry has led to the release of a variety of toxic substances, which harm the environment and have adverse effects on human health. For example, the burning of fossil fuels produces the sulfur and nitrogen oxides which cause acid rain, and also produce the carbon dioxide which causes global warming. The incineration of wastes produces dioxins which harm human health, and nuclear power generation produces radioactive wastes that must be safely stored for thousands of years. Unfortunately, there is no established method for discussing the impacts of different types of environmental toxicants together, and there are no methods for comparing the degree of their impacts each other. For example, scientific knowledge is insufficient to correctly compare the environmental impacts of thermal and nuclear power generation.

This research group aims to develop scientific methods for assessing and comparing the impacts of radioactive substances and other environmental toxicants, and to create a safe environment, under the "Comparative Study of the Effect of Radiation and Other Environmental Risk Sources on People and Ecosystems". These activities also provide basic information on environmental radiation protection, which is increasingly becoming a worldwide concern. The group consists of four research teams: Environmental Behavior Research Team, Experimental Model Ecosystem Research Team, Environmental Toxicology Research Team, and Numerical Analysis and Computer Simulation Research Team. The following describes the progress of each of these teams during 2003-2004.

Progress of Research:

Environmental Behavior Research Team:
This team investigates the levels and behavior of environmental toxicants in natural and semi-natural ecosystems, such as forests and farmland. To obtain the parameters which will enable the behavior of radionuclides and other environmental toxicants such as heavy metals to be compared, environmental samples (e.g., soils, plants, mushrooms, and earthworms) are analyzed for more than 40 elements, as well as for radionuclides. The role of biological activities on the behavior of radionuclides and related stable elements in ecosystems is one of the primary concerns. This team is also developing simple, accurate methods for analyzing long-lived radionuclides, such as technetium, plutonium, and

![Fig.2 Relationship between stable Cs and $^{137}$Cs in biological samples collected in two different forests, Babchin and Korenevka, in Belarus (Yoshida et al. 2004).](image)
uranium, in environmental samples.

Forests are important ecosystems in the terrestrial environment, and are one of the team's research targets because they tend to accumulate radionuclides discharged into the atmosphere through nuclear weapons testing and nuclear accidents. As the chemical behavior of radionuclides is expected to be almost identical to that of stable Cs, analyses of stable cesium (Cs) and related stable elements should be useful in gaining an understanding of the long-term behavior of radionuclides and its equilibrium distribution. Fig.2 shows the relationship between $^{137}$Cs and stable Cs in biological samples collected in 1998 in two forest sites with different contamination levels in Belarus. Even though several different species and parts of the same species were included, the concentration ratios of $^{137}$Cs to stable Cs were fairly constant for samples collected at the same forest site. This finding suggests that $^{137}$Cs, mainly deposited in forest ecosystems as a result of the Chernobyl accident in 1986, were well mixed with stable Cs within the biological cycle in the forest ecosystems by 1998. The transfer factor for each biological sample of $^{137}$Cs was almost the same as that of stable Cs, when calculated based on concentrations in the organic soil layer. This suggests that the stable-Cs-based transfer factor could be used as equilibrium transfer factor of $^{137}$Cs for many different types of biological samples in the forest.

**Experimental Model Ecosystem Research Team:**

A common index applicable to ecological toxicity is needed for a comparative evaluation between the effects of ionizing radiation to environmental biota and ecosystem, and those of other environmental toxicants. Ecosystems consist of various kinds of organisms, and have various characteristics that can be used as endpoints for the evaluation of ecological effects. This team has proposed an index for the holistic evaluation of effects on various ecological parameters. This ecological effect index (EEI) represents differences in values of applicable parameters between exposed and control ecosystems by the Euclidean distance function weighted by the ecological importance of each parameter. To demonstrate the usefulness of this index, we analyzed ecotoxicological data using the EEI for the effects of gamma-rays and other toxicants on a microcosm consisting of three microorganisms. The results showed that the EEI was positively correlated with doses of each toxic agent, and the relationship between them could be fitted by a sigmoid curve. From this curve, a 50-percent effective dose for the microcosm (ED$_{50}$), at which the EEI became 50 percent, could be obtained for each toxic agent. In conclusion, the EEI can holistically represent the effects of toxicants on various endpoints in model ecosystems. Since the ED$_{50}$ is a useful index for quantitative comparison of effects on model ecosystems between ionizing radiation and other toxic agents, it is expected that it will contribute to the comparative evaluation of effects on natural ecosystems.

In another study, the effect of toxic agents on the material flow in model ecosystems was investigated using a $^{13}$C tracer. *Daphnia magna* was exposed to radiation and cultured in a medium containing phytoplankton, which was previously labeled with $^{13}$C in the form of sodium bicarbonate. The concentrations of $^{13}$C in the *Daphnia magna* exposed to radiation were lower than the control, which was considered to be due to a lower intake of phytoplankton by inactivated *Daphnia magna*. This result indicated that the material flow in an ecosystem could be affected by radiation, and that the change in the carbon flow could be used as an indicator of ecological function.

**Environmental Toxicology Research Team:**

This team is comparing the relative risks of radiation and other environmental toxicants, using colony-forming abilities and damaged DNA in animal cells as an index. It is now investigating heavy metals and chemicals responsible for environmental pollution, which are compared with the risks of radiation.

It has been reported that antimony and arsenite inhibit the repair of radiation-induced DNA double strand breaks (DNA-dsbs), but it is not well known whether or not antimony induces DNA-dsbs. DNA-dsbs are induced by radicals, whose generation is modulated by intracellular glutathione (GSH). It is not obvious whether antimony induces the generation of radicals. In the current study, the team investigated the effect of GSH depletion on the colony-forming ability and DNA damage of Chinese hamster ovary cells (CHO), by treatment with antimony and arsenite. The cytotoxicity of antimony evaluated by colony-forming ability was the same level as arsenite in cells in which the level of intracellular GSH is normal. In GSH-depleted cells induced by treatment with buthionine-sulfoximine (BSO), cytotoxicity was markedly intensified by antimony, but not by arsenite. The repair of DNA-dsbs in GSH-depleted cells was also inhibited by a lower level of antimony. These experimental results suggest that the cytotoxicity of antimony is more sensitive to changes in the intracellular GSH level than arsenite.
The effects of quinone (a metabolite of benzene) on colony-forming ability and DNA-dsb in CHO and xrs-5 (DNA-dsb repair deficient mutant cell) were also investigated. Colony-forming ability was inhibited at a lower level of quinone in xrs-5 than CHO, and DNA-dsbs were induced by exposure of quinone.

In a number of studies, DNA damage-induced apoptosis has been reported to be dependent on p53. The team compared apoptosis induced by arsenite with radiation in the thymocytes of p53 knockout mice. Significant apoptosis was induced by irradiation in the thymocytes of normal mice. The extent of the apoptosis in p53 (+/-) mice was moderate, and no significant increase with radiation dose was seen in p53 (-/-) mutant thymocytes. Apoptosis induced by exposure to arsenite increased in p53 (-/-) mutant thymocytes as well as in normal mice. These findings suggest that DNA damage does not contribute to apoptosis in the cytotoxicity of arsenite.

Numerical Analysis and Computer Simulation Research Team:
The behavior of environmental toxics and their effects on ecosystems are complicated and diverse, and cannot be fully understood using experiments and surveys of natural ecosystems alone. This team is developing a computer simulation model based on accumulated data on the behavior of toxics in the environment, and their effects on ecosystems and living organisms. Another goal of the team is to contribute to protecting the environment from the effects of ionizing radiation, by developing a methodology for evaluating the radiation exposure of non-human species. It is also developing a mathematical model and computer simulation code to project the impact on the populations and communities of non-human biota.

The population dynamics, and mass and energy budgets, of an aquatic microbial ecosystems collected by other research teams are being simulated in a microcosm. A particle-based model has been used to duplicate this microcosm's self-organized, sustainable system of complexity, by simulating interactions among species, such as the predator-prey relationship, competition for common resources, autolysis of detritus and the detritus-grazing food chain, and interactions among organisms and habitats. Chronic, acute exposures to radiation and chemical toxics by the microcosm are being observed experimentally, and the results will be reflected in modifications to the simulation model. The goal in this is to define the protocols for determining the extent of the harm threatening whole species or creating imbalances between species, and thereby affecting the sustainability of the ecosystem.

Validity of this model is checked using data from the microcosm experiments. In the analysis, the intrinsic parameters of umbrella endpoints (lethality, morbidity, reproductive growth, mutation) are manipulated at the individual level, and the team is trying to determine the population-level, community-level, and ecosystem-level disorders of ecologically crucial parameters (e.g., intrinsic growth rate, carrying capacity, variation, etc.) that relate to the probability of a population's extinction. Numerical analysis and computer simulations will help us to compare the effects of various environmental toxics, and to develop and implement measures to protect the environment.

Major publications:
3.5. Studies on Environmental Radon and Its Biological Effects

Outline of Research Career:
Dr. Yamada received a Ph.D. from Nagoya University in 1989 for his study on collection performance of high efficiency particulate air filter. He has had 25 years of experience in research on radioactive aerosol and its internal exposure at NIRS. Between 1986 and 1987 he was at the Inhalation Toxicology Research Institute (ITRI) of Lovelace foundation, USA as a visiting scientist where he studied aerosol deposition within respiratory tracts using a cast model. He was awarded for studies on air filter by Japan Health Physics Society in 1986 and Japan Association of Aerosol Science and Technology in 1997.

Contact point (E-mail): yj_yamad@nirs.go.jp

Objectives:
Radon is a radioactive gas emanated from soil, water and building materials. Radon and its decay products in the air are inhaled into the human respiratory system where their further decay results in exposure. The alpha radiation, emitted from the decay products, has the potential to damage DNA of respiratory tissues, which would be the first step to cancer. It is well known that exposure to high radon concentration causes lung cancer from the results of many epidemiological and experimental studies. However, it has not been clear whether long-term exposure to environmental radon causes similar health effects. The radon levels in most homes are much lower than those in most uranium mines.

Among sources of natural radiations, radon and its decay products contribute the largest percentage to the total average annual effective dose to the public. There are two different ways to estimate dose from radon exposure; the epidemiological approach and the dosimetric approach. Currently, there is a large difference by a factor of 3 in exposure dose. The data on behavior in the environments and dose estimation for thoron, one of the radon isotopes, are very limited.

The aims of this research are to investigate the behavior of radon and thoron in the environments discriminatively, and to re-characterize their decay products for dose evaluation. This information would lead to a solution of the problems in risk estimation from exposure to radon and help to re-evaluate the dose conversion factor (DCF) from exposure concentration to exposure dose.

Progress of Research:
Studies with different approaches to effects of radon exposure in the environments have been carried out so far. This year, research activity was focused on solutions to the problems relevant to dose evaluation of radon exposure. Particle size distribution of radon decay products is one of the most significant factors regarding the dose evaluation. In this subject, a quick measurement method with a newly designed system of a graded screen array was developed. The radon and thoron concentrations with their particle size distribution were investigated in the Chinese Loess Plateau where epidemiology studies have been conducted until now. Moreover, research on measurement of radon concentration in water was also carried out. To study on biological effects, an experiment with culture cells was carried out. Because quality assurance in the radon measurement is an important subject, an intercalibration experiment with an institute in Germany was performed to obtain international traceability. In addition, a study on development of advanced technology for a radon trap was carried out.

Main subjects of studies carried out in this year are summarized below.

1) Particle size distribution of radon decay products
From the viewpoint of a dosimetric approach, the activity-weighted particle size distribution of radon decay products is one of the most important physical parameters for accurate dose evaluation of radon exposure. The National Research Council has demonstrated that the dose per unit exposure for inhaled unattached radon decay products is about 25 times higher than that for attached ones. A new system with a graded screen array was designed for measuring the particle size distribution of unattached...
radon decay products. Use of fine wire mesh screens achieved both a high volumetric airflow rate and high alpha count rate. Consequently, this improvement produced both a high sensitivity and good precision in particle size distribution measurement for unattached radon decay products with an activity median diameter around 1 nm. From the radon/aerosol chamber experiments, the particle size distribution of unattached radon decay products was observed at around 1 nm as a narrow peak with the geometrical standard deviation of 1.1. (Fig.3)

![PAEC](image)

**Fig.3.** Particle size distributions of radon decay products. The concentrations are 150 ± 29 Bq m⁻³ (black triangles), 590 ± 45 Bq m⁻³ (open circles), and 2377 ± 68 Bq m⁻³ (black circles).

2) **Field survey**

Comprehensive natural radiation measurements were carried out in cave dwellings widely distributed in the Chinese Loess Plateau. Those dwellings are located in Gansu Province. Radon and thoron gas concentrations were measured using a passive integrating radon-thoron discriminative detector. Thoron decay products concentrations were estimated from their deposition rate measurements. In particular, the particle size distribution measurement was made using a diffusion battery in some houses. Fig. 4 shows a typical particle size distribution of ambient aerosols. Two peaks were often observed below 20 nm and around 100 nm in such dwellings though the number concentration was low with a few thousand numbers/cc. Since the dose conversion factor depends on the particle size of radon decay products, this fact implies that the dose could increase significantly with such a small particle size.

3) **Quality assurance in the radon measurements**

For determining radon concentration in the atmosphere, there is no reference institute in Japan, and no method is set as a national standard regulated by the Japanese Industrial Standards. However, the gas storage ionization chamber method has been historically regarded as a standard method for radon measurements by many Japanese institutes.

The Physikalisch - Technische Bundesanstalt (PTB), the German National Institute for the Science and Technology is one European authority for metrology. Excluding France, many European institutes have traceability on radon measurements with the PTB directly or indirectly. We did a radon intercalibration experiment at the PTB. The PTB prepared radon gas in a chamber as the reference atmosphere and the NIRS estimated the radon concentration with the ionization chamber method. The intercalibration experiment provided important information on quality assurance of radon measurements at NIRS.

4) **Radon trap technique**

Radon is noble gas and chemically inert. However it seems that radon reacts with fluorine to form radon fluoride although the compound is not properly characterized. Based on the idea that radon can be adequately excited with a highly reactive property, radon might form some fluoric compounds. We confirmed of chemical reaction between radon and fluorine when a corona discharge was used as a promoter. The reaction was reversible, and the radon fluoride was stable only under the discharge field. Applying this phenomenon to a radon reduction technique, we developed a radon trap device using tetra-fluoric carbon (CF₄) gas. The trap efficiency of the device was over 99% at CF, of 5%. When the corona discharge was stopped, the trapped radon
was immediately released. The mass balance between trapped and released radon was reasonable and the trap performance was confirmed.

5) Radon in water
It has been reported that high radon concentrations can occur in water supplies from groundwater. Measurements of radon in water have been conducted by many investigators so far. While liquid scintillation counting is widely used for radon-in-water measurements in Japan, there are other available devices such as IM-fontactoscopes and atmospheric radon monitors with bubbling kits. In the present study, an intercomparison exercise was conducted for four devices for radon-in-water measurements. There was good agreement among the measured values (differences were within ± 3 %) for other devices than the IM-fontactoscope. The values measured with the IM-fontactoscope deviated from other measurement values. Since IM-fontactoscopes are used at some institutes in Japan even nowadays, it is necessary to check values measured with them for determination of radon-in-water concentrations.

6) Exposure of cultured cells
One of the most important problems for evaluating biological effects of radon exposure is to establish a method for estimation of the accurate absorbed dose. To consider the actual absorbed dose, exposure conditions for tracheal epithelial cells in vivo were reconstructed as an in vitro exposure system using an air-liquid interface culture (ALI culture). In the ALI culture, the apical surface of epithelial cells is not covered with culture medium. So the cells can be exposed to the short-range alpha rays of radon under the same conditions as in vivo. For rat tracheal epithelial cells, the dose response of ALI culture to X-rays proved to be the same as that for in vivo conditions. This result proved that ALI culture will be one of the most useful methods to facilitate future studies for investigation of the biological effects induced in tracheal epithelial cells by radon exposure.

Major publications:
3.6. Research on Redox Regulation against Radiation

Nobuo Ikota, Ph.D.
Director, Redox Regulation Research Group

Outline of Research Career:
Dr. Ikota was born in Saitama in 1947 and received B.S.(1971) and Ph.D.(1976) degrees from University of Tokyo. After working as a postdoctoral fellow (1976-1978) at Cornell University, he joined the Faculty of Pharmaceutical Sciences, University of Tokyo as an Assistant professor in 1978. In 1982, he joined to the National Institute of Radiological Sciences. His research interest is the development of antioxidants and radioprotectors, and the elucidation of thier defense mechanism.
Contact point(E-mail): ikota@nirs.go.jp

Objectives:
The redox (reduction and oxidation or oxidoreduction) processes have an important role in the physiological regulation of living organisms. Reactive oxygen species (ROS), reactive nitrogen species (RNS), and free radicals are produced in vivo when organisms are exposed to stresses from external factors such as radiation or ultraviolet light. The living organisms usually maintain homeostasis through their own control systems to remove ROS, RNS, and free radicals. However, oxidative stresses arise from insufficient removal of these species and cause various diseases such as arteriosclerosis, cancers, and aging. Redox regulation protects the living organisms from various oxidative stresses and maintains homeostasis by controlling the redox states in vivo. The redox group conducts studies on redox regulation research for biochemical effects from molecular, cellular, and tissue levels to the whole-body level through the participation of ROS, RNS, and free radicals generated by radiation. The research includes studies on bioradicals (development of the method to detect radicals such as hydroxyl radical (·OH), peroxyl radicals (LOO·), and nitric oxide (NO) generated in vivo by radiation), studies on biological effects by radiation (detection of oxidative damages of DNA, protein, and lipid, and elucidation of regulatory mechanisms on self-mutagenic and inducible genes and dysfunction of proteins, and radiation effects on endocrine systems), and studies on redox regulation substances (development of antioxidants, radical scavengers, and radioprotectors from synthetic compounds, natural products, and medicines, and elucidation of their defense mechanisms against ROS, RNS, and free radicals).

Progress of Research:
The Redox Group consists of four teams, which investigate the following topics.

1) Studies on bioradicals generated by radiation.
We have detected N-tert-butyl- α-phenylnitrene (PBN)-CH₃ adduct in the bile of rats injected intraperitoneally with a dimethyl sulfoxide solution of PBN and irradiated with X-rays (Fig. 6). It was confirmed using a scavenger of hydroxyl radical (·OH) that the PBN-CH₃ adduct detected in the bile of the rats is derived from secondary methyl radical formed by the reaction of solvent dimethyl sulfoxide and ·OH. This method was applied to show the ·OH scavenging activity of cysteamine and bunte salt.

In vivo ESR signal intensity in the abdomen of

Fig.6
In vivo monitoring of hydroxyl radical generation caused by X-ray irradiation of rats using the spin trapping/EPR technique.

mice injected with N-acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrrolidine (ACP) increased by the administration of sodium nitroprusside, a NO
generator. This finding suggests that ACP may be applicable to in vivo detection of NO production.

Basic in vitro study of spin trapping with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and 5-(diethoxypyridyl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) revealed that only DMPO enhanced the formation of ·OH from O₂. Both DMPO and DEPMPO were proved to permeate through lipid bilayers. The ESR spin trapping technique and scopoletin fluorescence spectroscopy, respectively, demonstrated that ·OH and H₂O₂ were generated dose-dependently in aqueous solution by the irradiation of X-ray or heavy ion particles.

2) Studies on regulatory mechanisms of self-mutagenic and inducible genes activated by radiation and fluctuation of cellular redox conditions.

We focused on both responsive genes and an endogenous retrovirus, intracisternal A-particle (IAP) with H-type long-terminal repeat (LTR), that is an endogenous mutagen and is activated by damages caused by radiation and /or ROS. The kinetic analyses were done by the measurement of exact amounts of these cellular RNA levels. The exactness of the method to quantitate RNA based on real-time reverse transcription polymerase-chain reaction (rt-RT-PTR) that was established in the last year was compared to the quantitative Northern blot hybridization. Both methods gave closely related data on the mean levels of RNA. On the other hand, the rt-RT-PCR method showed advances on both convergence and reproduction of data with a standard deviation (SD) lower than 10%, though the Northern method gave the SD of approximately 20%. Using the rt-RT-PTR, we found that cysteamine, an antioxidative radioprotector, activated the expressions of both heme oxygenase 1 (HO-1) and junB gene in murine macrophage cell line RAW264.7. The quantitative rt-RT-PCR method was further improved for specificity as well as accuracy. Using the improved method, levels of RNA for the IAP with H-type LTR in the presence of closely related RNA fragments were successfully quantified. Selective and continuous activation of the IAP with H-type LTR in hematopoietic cells in C3H/He inbred mice was revealed. The cellular metabolic systems for ROS have potential to modify the radiosensitivity, since living cells are damaged by the reactive oxgens generated by low LET radiation. To study the effect of the metabolic enzymes on cellular damage by radiation, cDNAs for glutathione peroxidase (GPx)1, GPx4, super oxide dismutase (SOD) 2 and SOD3 were isolated. We constructed a series of expression vectors for these genes from modifications of amino acid sequences. Cell lines were established by the stable transfection of these vectors into the RAW264.7 cell line.

3) Radiation effects on endocrine systems.

We demonstrated previously that administration of NO scavenger or NO synthase inhibitor decreased the incidence of mammary tumors of rat irradiated with gamma-rays. Thus NO might participate in radiation-induced tumorigenesis. Subsequently, we examined the effects of ionizing radiation on the production and action of NO in the epithelium of mammary glands using a mouse mammary epithelial cell line (HC11). In the culture medium of HC11 cells, an elevation (3-5 fold) in the concentration of nitrite that appeared to be derived from the oxidation of NO produced by the cells was detected with Griess reagent after X-ray irradiation (~30 Gy) of the cells. However, this elevation was not inhibited by treatment with inhibitors of NO synthase (NOS), such as Nω-monomethyl-L-arginine (L-NMMA) and S-methyl- L-thiocitrulline (SMTC). Therefore, it is plausible that the nitrite is not derived from the NO produced by the iNOS mediated pathway in mammary epithelial cells, and/or that epithelial cells may not be a source of NO, and other populations, such as myoepithelial cells, may contribute to the production of nitrite following X-ray irradiation in the mammary gland.

On the other hand, to elucidate the role of NO in cellular and molecular events, we examined the effect of NO on the expression and localization of tight junction-associated proteins in a mouse mammary epithelial cell line (HC11). Continuous localization around the cell perimeter of occludin and ZO-1, components of a tight junction, was disorganized, and descending expression of these tight-junctional proteins was revealed in HC11 treated with NOC18, a NO donor. Tyrosine phosphorylation of several proteins was induced in a non-ionic detergent insoluble fraction of the cells incubated with NOC5, another NO donor. Furthermore, immunocytochemical observation for the phosphorylated proteins in the NOC5 treated cells showed distinct labeling at cell-cell junction sites. These results suggest that NO influences expression and localization of tight junctional proteins in mammary epithelial cells, and that the phosphorylation of these proteins may participate in functional modification of tight junctions.

4) Studies on damages of cellular components and dysfunction of proteins by radiation and ROS, and redox regulation substances.

To estimate the oxidative DNA damage by X-ray
irradiation, the level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in extracted cellular DNA was measured by HPLC with electrochemical DNA detector. The formation of 8-OHdG increased dose-dependently in a range from 0 to 400 Gy. The change of activity for antioxidative enzyme such as SOD and GPx by radiation was also investigated. The GPx activity (2 hours to 4 day) in C3H/He male mouse liver by whole-body irradiation (X-ray 15 Gy) decreased compared with the non-irradiation group, while there were no significant differences for SOD activity. This result shows that the radiosensibility of GPx is higher than that of SOD. The dysfunction of protein by peroxynitrite(PN) and the development of scavengers against PN were studied. It was demonstrated that nitration of cytochrome c by PN resulted in the suppression of the activity of apoptosisome complex (caspase-9/Apaf-1/cytochrome c). The involvement of other apoptotic factors in nitration-dependent cytochrome c inactivating for the apoptosis-cascade execution was investigated, and Smac/DIABLO, another apoptogenic factor inactivating IAP (inhibitor of apoptosis), was found to be released from mitochondria after the continuous PN treatment, suggesting that Smac/DIABLO response was not modified after PN treatment, and was potentially apoptogenic. In the light of these results, it was suggested that cytochrome c nitration after continuous PN exposure affected the function of the apoptosisome complex directly, and resulted in inactivation of the caspase-9 dependent caspase cascade.

An explorative investigation for PN scavengers was also performed, and it was found that several indole derivatives had a specific activity for the inhibition of nitration reaction by PN. This result implies that a new pathway of nitration by PN other than the caged radical pathway should be proposed.

The radical-scavenging mechanism of phenolic natural antioxidants has been investigated in O2-saturated propionitrile at 203 K by means of electron spin resonance. Artepillin C, a major component of Brazilian propolis, was found to scavenge cumylperoxy radical via a one-step hydrogen atom transfer. On the other hand, the scavenging reaction of cumylperoxy radical by (+)-catechin or quercetin proceeds via an initial electron transfer followed by proton transfer. One-electron oxidation potentials of (+)-catechin and quercetin determined by the second-harmonic alternating current voltammetry indicated that the more negatively shifted the one-electron oxidation potential was, the faster the radical-scavenging reaction became.

**Major publications:**


3.7. Basic Study of Radiation Hazards

Isamu Hayata, Ph.D.
Director, Radiation Hazards
Research Group

Outline of Research Career:

Dr. Hayata worked for three years at Roswell Park Memorial Institute in New York State as a research fellow. He received a Ph.D. from Hokkaido University in 1976 for his study on unusual Ph1 translocation in human chronic myelocytic leukemia. His major works at NIRS during 28 years are: 1) Development of cytogenetical methods and of automated system to detect the effects of low dose radiation, 2) International collaborative studies on chromosome aberrations in high background radiation areas, 3) Multidisciplinary research and management under Nuclear Cross-over Research Project in collaboration with other six national institutes, 4) Biodosimetry of the accidentally exposed persons such as those in JCO Tokaimura criticality accident, 5) Cytogenetical study on the genesis of radiation-induced mouse leukemia.

Contact point (E-mail): hayata@nirs.go.jp.

Objectives:

This research group aims at the overall investigation of radiation hazards at the levels of a molecule, cell, tissue, organ, and an individual. The group consists of four teams. Each team's major subjects are: cytogenetics and cytometry (first team), hematology and teratology (second team), molecular biology (third team), and proliferation and differentiation (fourth team). Objectives are as follows.

First team

The analysis of radiation-induced DNA damage, including chromosome aberrations, produces useful information about the effects of radiation on the human body as well as dose estimation. The first team has worked to establish accurate and speedy systems for chromosome analysis using up-to-date techniques of electronics and biotechnology. Recently, we constructed a more cost-effective and flexible metaphase finder system than before. This system is indispensable for automated chromosome analysis.

Second team

The most remarkable effects of radiation can be detected in hematological tissues. Adaptive response and bystander effect are two important phenomena among radiation effects on embryogenesis in radiobiology with a critical impact on novel biorepsonse mechanisms and risk estimates. The second team has been analyzing hematological changes in irradiated mice and effects of radiation in irradiated mouse fetuses.

Third team

The third team studies molecular mechanisms underlying radiation hazards, with the aims of establishing a scientifically justified risk assessment of radiation and of further improving radiation medicine.

Fourth team

The fourth team elucidates the mechanism of the effects of radiation on the proliferation and differentiation of mammalian cells at cellular and molecular levels.

Fig. 7.
Metaphone finder system
**Progress of Research:**

**First team**

Chromosomes of lymphocytes of the residents in a high background radiation area (HBRA) in the southern China, where the level of natural radiation is 3 to 5 times higher than a control, were studied in collaboration with the Chinese National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention. The effect of radiation was detected in the yield of dicentrics but not in that of translocations. Since dicentrics and translocations are equally induced by radiation, it can be concluded that the effect of radiation 3 to 5 times higher than the normal level is not significant compared with that of other elastogens such as chemicals and metabolic factors (active oxygen species) that affect the human body under normal living circumstances. Further study on the effect of smoking as one elastogen is being conducted.

For biological dosimetry by counting chromosomes aberrations, automation techniques are required to process a large number of sample preparations at low dose radiation exposure. Metaphase Finder is an optical microscope system, which automatically scans and finds metaphase cells on the slide glass, and relocates metaphase cells to the center of the field of view of the microscope to observe chromosomes in high magnification. There are several commercial products of metaphase finders, but these dedicated-system products are usually expensive and inconvenient for fitting to the variety of conditions of sample preparations.

Now, we have constructed an improved system by assembling each component of the system, such as the microscope, automated stage and computer, by selecting from commercially available products, instead of purchasing one dedicated system. The new system has high cost-effectiveness and high flexibility in adapting to the new staining methods, such as chromosome painting.

This Metaphase Finder system consists of the following components: an optical microscope, a motorized sample stage, an auto-focusing unit and two CCD cameras for focusing and for image acquisition. These components are controlled by a personal computer. The image recognition software for detecting metaphase cells from the microscope image was developed on the programming environment provided by general-purpose image analysis software.

The processing speed of this Metaphase Finder system is 20 to 30 minutes per slide glass (typical scan area is 20 mm x 50 mm), which is sufficiently acceptable for practical use and remarkably improved from the speed of our previous model of 1993. The algorithm for detecting metaphase cells has also been improved to adapt to the variety of conditions of sample preparation.

This new system is scheduled to be distributed to several laboratories in Japan, where it will be tested for practical use.

**Second team**

The evidential correlation and interaction between two phenomena of adaptive response (AR) and bystander effect (BE) were observed in cultured limb bud cells. AR was induced by conditioning irradiation (0.3 Gy) of the E11 cells resulting in a significant protective effect against the occurrence of apoptosis, inhibition of proliferation and differentiation induced by a challenging dose of 5 Gy on the next day. Both protective and detrimental BE were observed, namely, irradiating 50% of the E11 cells with 0.3 Gy led to a successful induction of the protective effect, and irradiating 70% of the

![Fig. 8](image)

Survival rates of cultured limb bud cells showing a detrimental bystander effect (A) and a protective bystander effect (B).
E12 cells with 5 Gy produced an equal detrimental effect when 100% of the cells were irradiated. Further, the BE was markedly vanished by blocking the gap junction-mediated intercellular communication. These results indicated that the BE played an important role in both the induction of a protective effect by the conditioning dose and the detrimental effect of the challenging irradiation (Fig. 8).

**Third team**

GADD45a gene is regulated by p53 after ionizing irradiation. Recently it was shown that p53-dependent activation of genes in human and mouse cells requires additional transcription factors such as Sp1, GKLK, Ets1, and IRF-1. To examine the possible involvement of cooperating factors in transcriptional regulation of the GADD45a gene by ionizing radiation, we comprehensively searched for the X-ray-inducible binding locus of nuclear factor throughout the upstream region (-2244 bp / +89 bp) and the third intron (+1389 bp / +2488 bp) of the GADD45a gene by EMSA using 136 probes. The X-ray-responsive binding of nuclear factors was detected at eight loci in human myeloblastic leukemia ML-1 cells. Oct, NF-κB, HNF, NF-AT, and KLF family transcription factors were identified by the competition assay. An EMSA revealing activation of a transcription factor Oct is shown in Fig.9 as an example. We conclude that these transcription factors are activated by ionizing radiation in ML-1 cells. It is possible that some of these factors cooperate with p53 to mediate transcriptional regulation of the GADD45a gene after ionizing irradiation.

![Fig.9](image)

**Fourth team**

To clarify what factors derived from keratinocytes are involved in regulating the proliferation and differentiation of epidermal melanocytes in pigmented spots long after the cessation of UVB irradiation, granulocyte-macrophage colony-stimulating factor (GMCSF) was supplemented to melanoblasts and melanocytes in keratinocyte-depleted cultures. GMCSF induced the proliferation and differentiation of melanocytes in keratinocyte-depleted cultures. Anti-GMCSF antibody supplemented to the media inhibited the proliferation of melanoblasts and melanocytes as well as the differentiation of melanocytes from UVB-induced pigmented spots of irradiated mice, but not from non-irradiated mice. Enzyme-linked immunosorbent assay of culture media revealed that GMCSF secreted from irradiated keratinocytes was much greater than that secreted from non-irradiated mice. These results suggest that GMCSF is one of the keratinocyte-derived factors involved in regulating the proliferation and differentiation of mouse epidermal melanocytes from UVB-induced pigmented spots (Fig.10).

![Fig.10](image)

Kinetics of the proliferation of epidermal melanoblasts and melanocytes derived from the control mice co-cultured with the UV-irradiated keratinocytes [UV(+)]K and the anti-GMCSF antibody. Pure melanoblasts/melanocytes in melanoblast-proliferation medium (MDMDF2) were cultured with UV-irradiated keratinocytes with melanocyte-proliferation medium (MDMD2) plus rabbit-IgG (○, control) or MDMD2 plus an anti-GMCSF antibody (●, 25; △, 250; ▲, 2500 ng/ml). In the anti-GMCSF antibody-treated culture, the number of melanoblasts and melanocytes (A) and melanocytes (C) dramatically decreased. However, the number of melanoblasts (B) did not decrease.
3.8. Analysis of Gene Networks in Response to Ionizing Radiation

Kouichi Tatsumi, M.D., Ph.D.,
Director, Transcriptome Profiling
Research Group

Objectives:
We have developed a high performance gene expression profiling technique called HiCEP and in 2003 we focused on achieving high throughput with this method. We also identified genes of interest.

Specific tasks:
1. Preparing a peak database
2. Achieving high throughput
3. Establishing a knockout mouse preparation system

Progress of Research:
Preparing a peak database: We decreased the number of false positive peaks from over 50% in standard AFLP to under 5%, enabling us to efficiently isolate peaks of interest and determine the relationship between peak and gene unequivocally. We began preparing a HiCEP peak database for the mouse embryonic stem (ES) cell line, detecting about 38,000 peaks and sequencing about 15,000 of them.

Achieving high throughput: HiCEP analysis consists of six steps:
1. RNA preparation
2. cDNA synthesis
3. Preparation of HiCEP template
4. PCR
5. Separation by capillary electrophoresis
6. Data analysis using bioinformatics
By refining these steps, we achieved a 10-fold improvement in performance.

*RNA preparation: Using an automatic RNA elution system six samples can be treated simultaneously, allowing us to elute the RNA fraction from up to 48 samples. The quality of RNA fraction obtained by this procedure was good enough to use for analysis.
* cDNA synthesis and Preparation of HiCEP template: If magnetic beads are used for DNA handling, HiCEP analysis can be carried out by machine. We made a prototype automated HiCEP analyzer that can analyze three reaction plates of 96 wells apiece per week. The reproducibility is extremely good, and we are now testing the machine.
*PCR: This step requires a highly controlled PCR machine, and the variation in temperature should be less than 0.2°C. Few available PCR machines can satisfy this requirement, so we are making our own prototype.
* Separation by capillary electrophoresis: We improved the throughput of this step by a factor of ten by using a 16 capillary sequencing machine. Now we are trying to achieve a throughput of 384 capillaries.

Establishing a knockout mouse preparation system: We created knockout mice for RecOL4 and RecOL1. The RecOL4 knockout mouse exhibits growth retardation and premature aging. This is the first example of a knockout mouse for a RecO helicase gene that represents early aging phenotype as shown in the following pictures.
3.9. Development of Experimental Animals for Research on the Biological Effects of Radiation

Satoru Matsushita, D.V.M., Ph.D., Director, Laboratory Animal Development and Research Group

Outline of Research Career:
Dr. Matsushita studies in the field of laboratory animal sciences. Major works are concerned with research for infectious diseases of mice and rats, pathological and physiological research for the already established and newly developed mouse and rat strains and research for biological effects of radiation using laboratory animals. He also manages the laboratory animals and laboratory animal facilities considering animal welfare and protection as well as ethics for animal experimentation. D.V.M., Ph.D., Diplomate of the Japanese College of Laboratory Animal medicine, Member of the Japanese College of veterinary pathologists; Contact point (E-mail): matu_sat@nirs.go.jp

Objectives:
The purposes of this project are to develop new biotechnology to establish genetically modified animals for research on the biological effects of radiation, to produce animals highly sensitive to radiation, and to establish genetically and microbiologically controlled laboratory animal systems. The following are the specific objectives of this project.

1) Establishment of techniques of intracytoplasmic sperm injection (ICSI) to apply for production of transgenic mice and cryopreservation of sperm.
   Development of reproductive biotechnology such as in vitro fertilization, oocyte maturation using inbred mice.

2) To establish a method of mutagenesis in medaka and to produce at least one strain of radiation-sensitive medaka

3) To improve the diagnostic technology for infectious diseases of laboratory animals including molecular biological methods, and to simultaneously collect and disseminate physiological and pathological data on newly and already established strains of laboratory animals

In order to accomplish these objectives, the following attempts were made in the year 2003.

1) To establish the techniques of ICSI to apply for production of transgenic mice and cryopreservation of sperm.

2) To develop reproductive biotechnology such as in vitro fertilization and oocyte maturation, using inbred mice.

3) To screen mutant medaka at the third generation for their sensitivity to radiation.

4) To establish diagnostic antigens using recombinant viruses in developing a highly specific serodiagnostic method for lactic dehydrogenase virus (LDV) infection, which is one of the major virus infectious diseases in small laboratory animals.

5) To complete the compilation of data on the sensitivity of 10 strains of mice bred at this institute to bacteria (cilia-associated respiratory bacillus) causing chronic respiratory diseases, to clarify the disease characteristics in laboratory animals used in the study of the effects of radiation.

Progress of Research:
Fiscal year 2003 is the third year in the Middle Range Research Plan; to date we have published ten original papers and made steady progress in this project. The followings are our research accomplishments in the year 2003.

1) Attempt was made to apply ICSI for injection of dormant sperm cryopreserved without any cryoprotectant. Comparison was made for development of ICSI embryos injected with sperm stored for 3 months and 1 year at various temperatures (-20, -80°C and in liquid nitrogen). For 3 months storage, developmental ability of ICSI embryos after embryo transfer was approximately 20% and temperatures during storage has no significant effect. However, for 1 year of storage developmental ability of ICSI embryos injected with sperm stored at -20°C (2%) was significantly lower than with sperm stored -80°C (15%) and in liquid nitrogen (19%). These results indicate sperm
cryopreservation at -80°C and in liquid nitrogen is appropriate for long (> 3 months) period of storage.

Fig. 12.
Intracytoplasmic sperm injection (ICSI) in mice (Left) Sperm head (dotted circle) is directly injected into the cytoplasm of an unfertilized ovum. Exogenous DNA can be injected with a sperm head to produce transgenic mice. (Right) In vitro cultured mouse blastocysts derived from ICSI embryos injected with DNA coding green fluorescence protein (GFP).

2) Effect of aging in vivo and in vitro on developmental competence of ICSI embryos was examined. Ova were injected with fresh sperm at 13, 16, 19 and 22 h after injection of human chronic gonadotropin (this hormone induces ovulation). In vitro development to blastocyst stage 120 h after ICSI was between 55-88% and there was no significant differences among aging periods tested both in vivo and in vitro.

3) Conditions of in vitro fertilization (IVF) were studied to improve fertilization in BALB/c mice. Attempt was made to improve in vitro capacitation of sperm. We found that isotonic osmotic pressure (305 mOsmol) and elimination of lactate results in good fertilization. Furthermore, Ca^{2+} was shown to have no significant effect on sperm capacitation unlike sperm penetration through the zona pellucida, which need high Ca^{2+} (>2.5 mM).

4) For the mutagenesis in medaka, male fish were treated with chlorambucil (0.2 mM) and the resultant mutations were recovered in the F_{3} progeny by the method of three-generation crosses: The treated males were pair-mated with untreated females to produce F_{1} fish. Each F_{1} fish was mated with an untreated partner to produce F_{2} family (F_{2} founder fish and the F_{1} progeny). When the F_{2} fish grew to adult stage, several single-pair crosses between siblings were performed for each F_{1} family to produce F_{3} progeny. The mutations for radiation sensitivity were screened in the F_{3} progeny by exposing them with X-irradiation at the dose of 2Gy. No effect has been found in normal medaka embryos at this exposure dose. We have completed the screening of 63 pairs from nine F_{1} families. It was found for one F_{1} family that approximately 25% of the embryos of the pair died following 2Gy X-ray irradiation. This medaka strain is considered to be a candidate mutant strain having a high sensitivity to radiation.

Fig. 13.
Medaka inbred strains: A) HNI strain B) i3 strain.

5) To establish a highly specific serodiagnostic method for LDV infectious disease, we carried out virus core protein and envelope protein expression studies using various expression systems such as Escherichia coli, insect cells and animal cells. The expression of LDV M/VP-2 was possible in insect expression systems using recombinant vacuolar viruses. The expressed M/VP-2 protein can be harvested in the soluble state and shows properties similar to those of the M/VP-2 protein originating from a virion; it is considered to be a useful antigen for diagnosis.

6) Regarding the collection of pathological data from laboratory animals, three strains of mice (B10, B10-Thyl.1, and B10.D2) bred at this institute were inoculated with cilia-associated respiratory(CAR) bacillus, and all strains of the inoculated mice presented with moderate sensitivity to the bacterium. The collection of pathological data from the 10 strains of mice bred in this institute was completed. Furthermore, we compared histopathological changes with time after infection between mice with high sensitivity (BALB/c-mui +/+ mice) and mice with low sensitivity (A/J mice). Compared with A/J mice, the attachment of the bacilli to the epithelial cells and the development of severe lesions in the airways in BALB/c-mui +/+ mice were observed in early stages.

7) Collaborative studies with the Transcriptome Research Center and the Transcriptome Profiling Group have been promoted. Experiments for the
production of gene-manipulated mice, in which groups of new genes were identified by studying their transcriptome profiles, were carried out. That is, we generated chimeric mice derived from embryonic stem (ES) cells, by manipulating three new genes (A, B, and C) using the aggregation method for ES cells and host embryos, a simplified and practical method, which does not require various complicated equipments. The percentages of chimeric mice were as follows: chimeras derived from A gene, 28.9% (71/264); B gene, 19.2% (14/73); C gene, 32.1% (17/53), respectively; and control, 72.1% (145/201). Chimeric mice were established four strains and one of these strains was confirmed to be a new gene-manipulated mouse lineage.

8) Regarding the collection of physiological data on laboratory animals, we are currently collecting basic anatomical data on four strains of mice bred in collaboration with the Laboratory Animal Development and Management Office and preparing for the release of data on one strain.

**Major publications:**


3) Hiromi Omoe, Katsuhiko Omoe*, Masahiro Sakaguchi*, Yousuke Kameoka*, Satoru Matsuhashita, Toshiki Inada*: Analysis of protein expression by mammalian cell lines stably expressing lactate dehydrogenase-elevating virus ORF 5 and ORF 6 proteins., *Comparative Immunology: Microbiology & Infectious Diseases*, 27, 81-92, 2004


3.10. Studies on Experimental Carcinogenesis Induced by Plutonium Compounds

Outline of Research Career:

Dr. Yamada received a Ph.D. on Veterinary Medicine from the Hokkaido University in 1988. He has conducted studies on biological effects of alpha emitters at NIRS. He was at the Life Sciences Division, Los Alamos National Laboratory, USA as a visiting scientist where he studied alpha particle-induced mutation in hprr locus from 1993 through 1995. He proceeded to Institute for Environmental Sciences as a senior scientist from 1999 through 2001.

Contact point (E-mail): yt_yamada@nirs.go.jp

Objectives:

The purpose of this research subject is to investigate the biological effects and cancer risks of internally deposited radionuclides, especially alpha-emitting plutonium compounds, by using experimental animals and in vitro alpha particle exposure systems. To clarify the cellular and molecular mechanism of high LET radiation-induced carcinogenesis, current studies include: 1) identifying target cells for the lung and bone tumors induced by inhalation and injection of plutonium, 2) establishing primary cell cultures of the target cells and cell lines from the tumor tissues, 3) analyzing radiosensitivity and biological effectiveness of alpha particle in the target cells, identifying gene mutations and chromosome abnormalities in bronchial and lung epithelial cells, 4) defining the role of genetic and epigenetic changes of oncogenes and tumor suppressor genes in the development of lung tumors and the influence of irradiation on which genes are targeted, and 5) determining variation in target cells for lung tumors relates to lung cancer susceptibility.

In the present study, cell lines were established from plutonium-induced lung and bone tumors, and the biological properties were characterized. In addition to the lung tumor cell line, tumorigenesities of immortalized and transformed epithelial cell lines from rat respiratory tract were examined in nude mouse transplantation assay. Primary culture cells from rat trachea and lung also were established for future study of radiation-induced transformation and tumorigenicity assay.

Progress of Research:

Establishment and characterization of tumor cell lines from Pu-exposed animals

Lung tumor (adenocarcinoma) of rat exposed to plutonium dioxide was cultivated in vitro in an attempt to establish cell line by utilizing explant and trypsinization techniques. The epithelial cell line (PuD2) exhibited multilayering and anchorage-independent growth in agarose medium. Positive reaction of surfactant apoprotein A in immunohistochemical staining indicated that the PuD2 cells were derived from TypeII alveolar epithelial cells. Chromosome analysis by Giemsa staining revealed trisomy of No.4 and a marker chromosome of No.11. Transplantation of the PuD2 cells into nude mouse (BalbC nu/nu) resulted in the formation of nodule with a diameter of 12 - 15 mm within two weeks.

An cell line (mOS) was also established from bone tumor (osteosarcoma) of plutonium citrate-injected mouse. The mOS cells were morphologically similar to spindle-shaped cells or polykaryocytes. Tartrate-resistant acid phosphatase (TRAP) staining indicated that the mOS cells partially originated from osteoclast. The mOS cells developed a nodule at the injection site of nude mouse by 8-9 weeks after implantation. Use of these cell lines will allow investigation of carcinogenic mechanisms of plutonium compounds and alpha-irradiation. (Chromosome analysis was supported by Dr. Minamihisasamatsu and Dr. Kohno)

Tumorigenesis assay of respiratory tract epithelial cell lines

Several respiratory tract epithelial cell lines established from normal rats were also transplanted to compare tumorigenicity at different stages of...
cancerous development. Although virus-immortalized SV40T2 cells and gamma ray-transformed RTiv3 cells could not be tumorigenic, chemical transformed benzo [a] pyrene-induced BP (p53 wild type), BP(P)Tu, BP130 and BP270 (p53 mutation) cells formed a nodule three weeks after the implantation in nude mouse. These BP cell lines were transplantable and resulted in a rapid growth within two weeks in the second implantation. The growth of PuD2 cells, p53 wild type, is extremely rapid in the nude mouse. These results indicate that the tumorigenicity of respiratory tract epithelial cell lines is dependent on their different stages of the carcinogenic processes from the initiation through promotion and leading to the progression. The p53 mutation, however, does not seem to play an important role in the tumorigenesis of rat respiratory tract epithelial cells. Association with other genes and the genetic alterations is under investigation. (Table 1)

**Establishment of primary cultures of rat tracheal and lung epithelial cells**

The purpose of this study is to develop a method of isolating primary Type II cells and tracheal epithelial cells from rat. Pneumocytes were isolated from lung of Wister rats by enzyme digestion and separated on isotonic density gradients. Type II cells rich fraction was recovered from the density range around 1.06 g/ml of the gradient. The Type II cells were identified by immunohistochemistry utilizing epithelial cell- specific rat cytokeratin 17 antibody and by alkaline phosphatase staining, and the purity was approximately 40% in the staining. Tracheal epithelial cells were collected after enzyme digestion and infusion of buffered solution through trachea. The Type II and tracheal epithelial cells could be subcultured in serum-free medium including epidermal growth factors on Mylar bottom dishes for alpha irradiation. This primary epithelial cell culture system will be useful for analysis of radiation sensitivity among the different target cells and mechanistic studies of early changes in the rat lung carcinogenesis initiated by alpha irradiation.

**Table 1. Summary of Characteristics of the Cell Lines**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Animal</th>
<th>Origin of Cell Line</th>
<th>Tumorigenicity*</th>
<th>Anchorage- Independent Growth* *</th>
<th>p53 (Exon 5-8)</th>
<th>Chromosome Aberration</th>
<th>Originator</th>
</tr>
</thead>
<tbody>
<tr>
<td>PuD2</td>
<td>Rat</td>
<td>Lung tumor, Pu-induced</td>
<td>Yes</td>
<td>Yes</td>
<td>Wild type</td>
<td>Trisomy No.4 Marker chromosome No.11</td>
<td>NIRS</td>
</tr>
<tr>
<td>mOS</td>
<td>Mouse</td>
<td>Bone tumor, Pu-induced</td>
<td>Yes</td>
<td>Yes</td>
<td>Wild type</td>
<td>A. Clement, Univ. Paris</td>
<td></td>
</tr>
<tr>
<td>SV40T2</td>
<td>Rat</td>
<td>Type II cell from neonatal rat lung, virus-immortalized</td>
<td>No</td>
<td>No</td>
<td>Wild type</td>
<td>J. L. Poncey, CEA</td>
<td></td>
</tr>
<tr>
<td>RTiv3</td>
<td>Rat</td>
<td>Primary tracheal cell, gamma ray-transformed</td>
<td>No</td>
<td>No</td>
<td>Wild type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>Rat</td>
<td>Fetal lung, in vitro benzo [a] pyrene-induced</td>
<td>Yes</td>
<td>Yes</td>
<td>Wild type</td>
<td>E. May, CEA</td>
<td></td>
</tr>
<tr>
<td>BP(P)Tu</td>
<td>Rat</td>
<td>Tumor in syngeneric rat inoculated with BP cell</td>
<td>Yes</td>
<td>Yes</td>
<td>Codon 130 AAG to AGG</td>
<td>E. May, CEA</td>
<td></td>
</tr>
<tr>
<td>BP130</td>
<td>Rat</td>
<td>Cultured BP cell</td>
<td>Yes</td>
<td>Yes</td>
<td>Codon 130 AAG to AGG</td>
<td>E. May, CEA</td>
<td></td>
</tr>
<tr>
<td>BP270</td>
<td>Rat</td>
<td>Cultured BP cell</td>
<td>Yes</td>
<td>Codon 270 GTT to TTT</td>
<td>E. May, CEA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tumorigenicity assay in nude mouse
** Colony formation in agarose medium
\[ \text{Not tested} \]

**Major publication:**