RERF update RERF

News & Views from the US–Japan Radiation Effects Research Foundation

Volume 4, Issue 2 Hiroshima & Nagasaki Summer 1992

Collaborative Research with Chelyabinsk Group to Begin

On 12 May 1992, RERF Chairman **Itsuzo Shigematsu** and Alexander V. Akleev, director of the newly created Ural Research Center for Radiation Medicine (URCRM), signed an agreement to collaborate on research in the areas of epidemiology and statistics, dosimetry, and medical follow-up (see *RERF Update* 3(1):1, 1991 for a description of the Techa River and Kyshtym incidents that caused large-scale radioactive contamination responsible for extensive population exposures in the southern Ural Mountains area).

Epidemiologic data obtained by the two institutions will be used to assess the comparative risks of chronic and acute radiation exposures—an effort that both parties realize may not materialize until some time in the future. In the meantime, RERF and URCRM will work closely to evaluate the similarities and differences between the atomic bomb survivor and Chelyabinsk data sets and will attempt to obtain missing or incomplete data. Protocols for specific research activities will be developed as soon as possible.

In the area of physical dosimetry, RERF has little to offer that is of direct use in the Chelyabinsk situation. But, activities developed under a Japan–Russia collaborative agreement will concentrate on determining worker exposures and health effects in the Mayak reprocessing and production facility, in collaboration with RERF for the epidemiologic and statistical studies. Dosimetric collaboration is being planned under the aegis of the Nuclear Safety Research Association, Tokyo (chairman: **Eizo Tajima**), and the Japan Atomic Industrial Forum, Tokyo (executive managing director: **Kazuhisa Mori**). RERF will concentrate on technology transfer in the field of biological dosimetry, ie, detection of chromosome aberrations and use of somatic mutation assays, by helping to develop the capability for these techniques at URCRM.

Future activities are foreseen in fields such as medical follow-up (mostly consultative efforts by RERF physicians) and, at a later stage, genetics and molecular epidemiology studies.

International workshop focuses on Chelyabinsk nuclear accident consequences

From 14–17 June, the Chelyabinsk International Workshop was held at George Mason University, Fairfax, Va, USA, cosponsored by the US Departments of Energy and Defense, to discuss possible collaborative research and assistance activities. At the workshop RERF Vice Chairman **J. W. Thiessen** discussed the recently signed agreement with the URCRM.

"The meeting was attended by a number of Russian administrators and scientists, all with impressive credentials and backgrounds, in addition to many American scientists who are

continued on next page

RERF Chairman Elected Fellow of Royal College of Physicians of London

At a ceremony held in early June, RERF Chairman **Itsuzo Shigematsu** was conferred with the title of Fellow of the Royal College of Physicians of London. Shigematsu, who is now serving his third four-year term as RERF chairman, and World Health Organization Director-General **Hiroshi Nakajima** are the first Japanese researchers to be honored in this way.

A graduate of Tokyo and Harvard universities, Shigematsu was director of the National Institute of Public Health's Department of Epidemiology from 1966–81, where he is professor emeritus.

Dating from 1518, the Royal College of Physicians was established by Thomas Linacre, noted scholar and humanist, who was one of Henry VIII's physicians. Originally instituted as a way of prohibiting nonlicensed physicians from practicing in the vicinity of London, in the modern era the college has bestowed fellowships upon physicians of special achievement. ❑

J. TAKAYAMA

Shigematsu with citation

In This Issue

Dreams and Reality

by J. W. Thiessen, RERF Vice Chairman & Update Editor-in-Chief

J. TAKAYAMA

Thiessen

Radiation has played a central role in my professional career, and, as we all know, what dominates your life during the daytime sometimes spills over into your dreams at night. Within the last few months I had two dreams that I found rather interesting if not disturbing, and that made me continue to think about them in the twilight zone between dreaming and awakening, and beyond.

The first one followed a discussion "in the office" between RERF's present chief of research and myself. The discussion centered upon radiation as a mutagen, and **Jim Trosko** restated rather clearly what now appears to be a widely accepted idea, namely, that radiation is a poor "point mutagen" but an excellent "deletion mutagen." We talked a little more about oncogenes and suppressor genes, and why it is more likely that radiation in the process of carcinogenesis acts via its impact on suppressor genes rather than through the "turning on" of oncogenes. Being a layman in this field, I continued to think about the implications of what I had heard, which must have set off my dream.

In this dream, I was arguing with someone (myself?) that because there seem to be more oncogenes than suppressor genes and because radiation appears to take "bites" out of both, it should actually "turn off" more oncogenes than suppressor genes. This was such a baffling thought that I continued to think about it—whether I wanted to or not. Is radiation actually preventing more cancers than it provokes? This thought struck me as so revolutionary that I immediately rejected it when I awoke.

Since then, I have not tried to address this again, because I—my dream—must be so clearly wrong. We all know that radiation is a carcinogen, effective in some cases (leukemia comes to mind immediately), not so effective in others (most cancers other than leukemia), and not at all effective in some (small-intestinal and rectal cancers). Ergo, at least in most cases, radiation does not seem to prevent cancer. So why bother about such silly thoughts as can only occur in dreams? Before long, one would be tempted to dream about the fact that radiation has a different effectiveness in different tissues, or—now we get into the realm of nightmares—why it doesn't seem to do anything at all in a few tissues. Ah, all this nonsense!

My second dream may have been stimulated by the preceding one. I had read in the *Journal of the National Cancer Institute* (15 April issue) that it has become clear that we (the *then* experts) have grossly overestimated the risk of asbestos-related cancer (ie, mesotheliomas). In 1978, US Health, Education and Welfare Secretary **Joseph A. Califano** predicted that about 17% of all cancers in future years would be caused by asbestos (according to my rough calculation, that amounts to an average of approximately 100,000 cancer deaths per year). At a 1981 Banbury Conference, this number was reduced to between 5000 and 10,000 asbestos-related cancer deaths per year. The most recent estimates indicate that, at most, 2000 mesothelioma cases occur every year. Where are the missing cases?

Most recently, in the 19 June issue of *Science*, **Philip Abelson**'s editorial addressed the "Exaggerated Carcinogenicity of Chemicals," quoting grossly overestimated cancer effects from exposure to butadiene.

The next night I dreamed about a newspaper article (in some unclear future), accusing the experts (us) of having grossly misled the public into believing that radiation is a dangerous carcinogen. Now, upon awakening, I immediately realized that this was also a nonsensical dream. One only has to read the latest ICRP recommendations to realize how much we know about radiation and how reliable our present estimates are. Could we be off by a factor of 10 or so? Nah, impossible! Anyway, no one would ever be able to demonstrate that we were *that* wrong!

Notwithstanding popular belief, I am convinced that dreams never come true. That's a good thing, because otherwise one really could become disturbed from time to time. I am glad that I have been able, through this medium, to relieve any anxieties I may have developed in my chosen profession. These kinds of dreams play the same role as dreams about terrible automobile accidents. They make you a safer driver.

I surely hope so. ❑

Chelyabinsk Collaborative Research

continued from page 1

interested in research collaboration," remarked Thiessen.

The meeting consisted of open discussions of the multitude of problems to be addressed and presentations on the investigations performed to date. Thiessen's presentation was followed by a lively discussion on the importance of avoiding duplication and fragmentation of activities that could result in a dilution of the effectiveness of the total collaborative effort. Thiessen proposed that, at least in the areas of epidemiology and statistics in which RERF has unique expertise and experience, involvement of other scientists should be closely coordinated with RERF, which he believes should play the role of "lead laboratory." He recommended an identical approach in areas such as dose assessment, radioecology, and environmental reclamation, ie, with one laboratory or closely related group of scientists acting as the focal point of the entire effort.

Further developments in these collaborative research endeavors will be reported in future issues of *RERF Update*. ❑

The SCID-hu Mouse as a Model for Human Radiation Biology

Implanting human tissues into mice lacking fully competent immune systems offers a unique opportunity to assess radiation risk in vivo.

by Seishi Kyoizumi and Mitoshi Akiyama, RERF Department of Radiobiology, Hiroshima

The radiobiological study of humans has been hampered by a lack of suitable in vivo experimental models. Of course, acute and chronic radiation effects in humans have been documented in the studies of atomic bomb (A-bomb) survivors and patients irradiated either by therapeutic intent or by accident. However, the information gained from these studies has been limited by the difficulties in estimating precise radiation doses and in obtaining biological samples for directly analyzing the processes of radiation-induced pathogenesis. In vitro, it has been possible to deduce simple survival data for human clonogenic cells such as skin fibroblasts, T lymphocytes, and bone marrow CFU-C* under defined conditions. However, it is not clear whether such in vitro conditions adequately reflect the complex microenvironmental conditions that exist in vivo. Given these limitations, meaningful risk estimates are difficult to achieve. With these issues in mind, we propose that the severe combined immunodeficient mouse–human (SCID-hu) chimera can be used as an in vivo experimental model for human radiation biology.

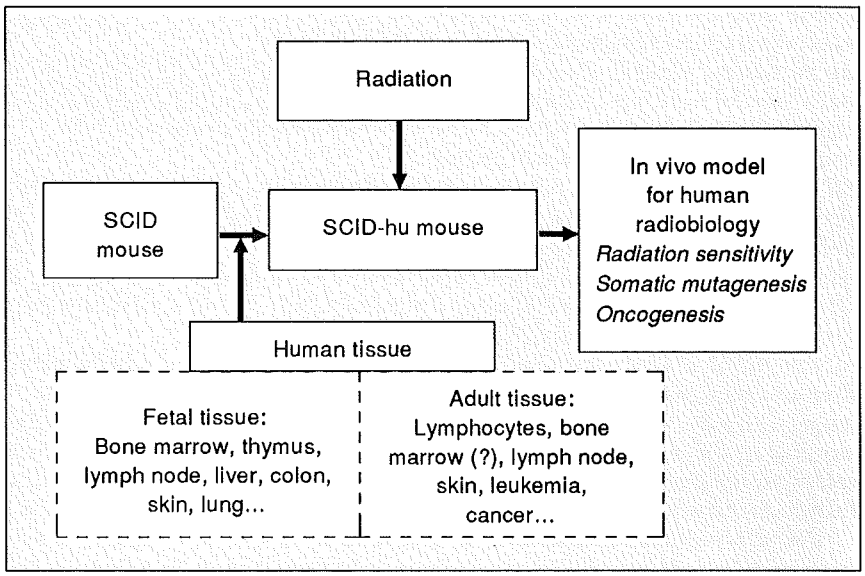


Figure 1. Applications of the SCID-hu mouse system to studies of human radiation biology.

The SCID-hu mouse model

In 1988, **J. M. McCune** and colleagues at SyStemix Inc, Palo Alto, Calif, succeeded in implanting functional human fetal hematolymphoid organs including thymus and lymph node tissues into the severe combined immunodeficient (SCID) mouse (JM McCune et al, *Science* 241:1632–9, 1988). Recently, we reported successful implantation of functional human fetal bone marrow into the mouse (S Kyoizumi et al, *Blood*, in press). Other human tissues such as skin, lung, and colon also were found to be transplantable and morphologically normal in SCID mice (personal communication with R Namikawa et al, SyStemix Inc, Palo Alto, Calif). Such a chimeric mouse (ie, the SCID-hu mouse) can be experimentally irradiated with any type and dose rate of ionizing radiation to analyze in vivo effects of radiation on human tissues (Figure 1).

As a result, it may now be feasible to conduct analyses of in vivo radiation sensitivity of human tissues, as well as studies in vivo of radiation-induced somatic mutagenesis such as specific gene mutations and chromosome aberrations. We also might be able to study transformation or oncogenesis in these irradiated human tissues followed by appropriate promotions. Using this experimental system, we may be able to answer many questions about the mechanisms for radiation-induced carcinogenesis (JE Trosko, *RERF Update* 4(1):3–5, 1992). Thus, it is expected that this SCID-hu model may provide complementary and supportive data for the studies of A-bomb survivors and accidentally exposed people. Since the conclusions from the study using this model would be drawn directly from observations of human tissues in vivo, it is likely that they will contribute more relevant and meaningful insights to human radiation biology than those obtained in vitro or from other nonhuman animal models.

Here we will summarize briefly the effects of radiation on human bone marrow engrafted into SCID mice. We also will discuss a possible application of this bone marrow model to the study of somatic mutations in hematopoietic stem cells of A-bomb survivors.

Effect of radiation on human hematopoiesis in the SCID-hu mouse

The SCID-hu bone marrow model was created by surgically implanting human fetal bone fragments (between the 18th and 22nd gestational weeks) into SCID mice (S Kyoizumi et al, *Blood* 79:1704–11, 1992). The advantage of implanting bone fragments is that not only hematopoietic stem cells but also the microenvironment required for hematopoiesis can be transferred to the mice. The implantation process was associated with an immediate decline in hematopoietic activity in the bone grafts, followed by a recovery. Histologic examination at 2–3 weeks showed necrotic

**Colony-forming unit–culture*

continued on next page

SCID-hu Mouse

continued from page 3

changes in the marrow and no clear foci of hematopoietic cells were observed. After 6–8 weeks, most of the grafts looked morphologically similar to normal human bone marrow: a high degree of cellularity was associated with human lymphopoiesis, myelopoiesis, erythropoiesis, and megakaryocytopoiesis. More than 90% of the bone grafts demonstrated and maintained such signs of hematopoiesis for as long as 20 weeks. Human progenitor cell activities for multiple lineages including myeloid progenitors (colony-forming unit–granulocyte macrophage, CFU-GM) and erythroid progenitors (burst-forming unit–erythroid, BFU-E) also were demonstrated in the methylcellulose colony assay. Flow cytometric analysis demonstrated that the cells recovered from CFU-GM and BFU-E colonies expressed human lineage-specific markers, CD15 and glycophorin A, respectively. Constant levels of progenitor activity within the range normally found in human bone marrow were maintained in the grafts for longer than 20 weeks. These findings demonstrate that a suitable microenvironment for maintaining human stem cells and for inducing their differentiation can be successfully introduced into SCID mice.

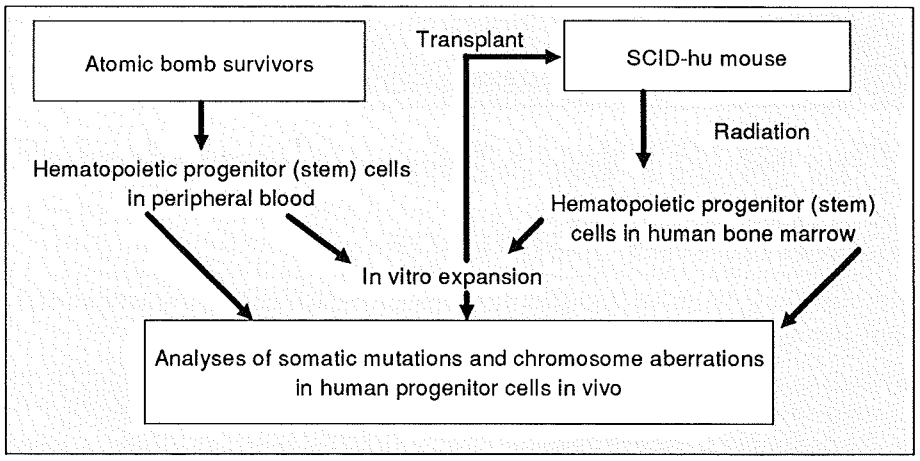


Figure 2. Strategies of studies on radiation-induced somatic mutations in hematopoietic progenitor cells.

Using this SCID-hu model, it is possible to derive quantitative data about radiotoxic effects on human hematopoiesis in vivo (S Kyoizumi et al, *Blood*, submitted). After whole-body X-irradiation, human fetal bone marrow implanted into SCID-hu mice showed a typical radiation sensitivity for mammalian hematopoietic progenitor cells. The survival curves for CFU-GM and BFU-E were found to have no shoulders, and the D_0 value was 1.0 Gy for CFU-GM and 0.7 Gy for BFU-E. This D_0 value of CFU-GM is similar to that found for human bone marrow CFU-GM irradiated in vitro, as well as for mouse CFU-S (colony-forming unit–spleen) and CFU-GM irradiated in vivo (JH Hendry, *Int J Radiat Biol* 47:3–16, 1985). Greater sensitivity of erythroid progenitors to radiation also has been reported for mouse and dog bone marrow. These data suggest that the radiobiological characteristics of human hematopoietic progenitor cells can be maintained in the bone grafts. Also, this X-ray–induced acute hematotoxicity was significantly reduced when the well-known radioprotective agent WR-2721 (a free radical scavenger) was administered before irradiation. The degree of the dose-reduction effect of WR-2721 was very similar to that reported for normal mouse bone marrow. Furthermore, after low-dose irradiation (less than 1.5 Gy of X-irradiation), hematopoietic functions in the bone marrow graft could recover to the normal level. Previous studies in human clinical trials have demonstrated that human granulocyte–colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) can promote hematopoietic recovery after myelosuppression caused by radiation exposure (RP Gale and A Butturini, *Exp Hematol* 18:958–64, 1990). We demonstrated that recovery of human CFU-GM activity in SCID-hu mice was accelerated by the administration of human G-CSF, and neutrophil populations remarkably increased within the bone marrow grafts. Thus, human G-CSF augmented myelopoiesis by stimulating myeloid progenitor cells that survived radiation exposure.

These studies have demonstrated that the SCID-hu model may prove suitable for risk analysis of human radiation exposure, as well as for developing new modalities for preventing and treating radiotoxic damage to the human hematopoietic system.

Applications to somatic mutation studies of A-bomb survivors

The SCID-hu bone implant model might be used to analyze other features of human bone-marrow radiobiology, especially radiation-induced mutations in hematopoietic progenitor cells of A-bomb survivors (Figure 2). An elevated somatic mutation frequency in erythrocyte glycophorin A genes was detected in A-bomb survivors more than 45 years after radiation exposure (R Langlois et al, *Science* 236:445–8, 1987; S Kyoizumi et al, *Cancer Res* 49:581–8, 1989; M Akiyama et al, *J Radiat Res* 32(Suppl):278–82, 1991). Also, common HPRT gene mutations and chromosome aberrations were found among various T- and B-cell clones isolated from the same survivors (M Hakoda et al, *J Exp Med* 169:1265–76, 1989; Y Kusunoki et al, RERF, manuscript in preparation). These observations suggest that radiation-induced mutations were recorded in bone marrow stem cells and that mutant stem cells continuously supplied mutant mature blood cells in A-bomb survivors. Because this conclusion was based on observations from analyses of mature blood cells, direct demonstration of such mutations in hematopoietic progenitor or stem cells is being sought eagerly.

One approach is to analyze somatic mutations and chromosome aberrations induced in progenitor cells isolated from SCID-hu mice after radiation exposure (Figure 2). Another is to isolate hematopoietic progenitor cells directly from the peripheral blood of A-bomb survivors. Recent advances in stem-cell technology make it possible to purify hematopoietic progenitor cells and to expand them in culture containing various hematopoietic growth factors including stem cell growth factor (ON Witte, *Cell* 63:5–6, 1990). Using this technique in studies of A-bomb survivors, we might be able to demonstrate and measure directly somatic mutations in hematopoietic progenitor cells circulating in the peripheral blood. Furthermore, these mutant progenitor cells might be transferable to SCID mice, where they might differentiate to mature blood cells in the mice after administration of various human hematopoietic growth factors. We hope these two approaches will systematically demonstrate the dynamics of generation and differentiation of mutant stem cells after radiation exposure. ❑

Is There a Gene Affecting Human Radiosensitivity on Chromosome 8?

Preliminary data using SCID mouse/human fibroblast hybrids provide fascinating evidence.

by Masahiro Itoh, Kiyohiro Hamatani, and Masumi Abe, RERF Department of Radiobiology, Nagasaki

Ongoing efforts to analyze the mechanisms underlying radiation effects and radiosensitivity are of great interest worldwide to both scientists and regulators who set radiation protection standards.

Clear evidence exists of radiosensitivity in rare human genetic syndromes, such as xeroderma pigmentosum and ataxia telectangia, which are caused by the presence of two defective genes. The prevalence of other less severe genetically predisposed radiosensitivity genes in the general population, and the impact of these genes in an individual carrying one normal gene in addition to a defective gene, as yet remain unknown.

At the RERF Nagasaki Laboratory, we are involved in attempts to isolate the normal genes involved in human DNA repair—though admittedly this is only one component of late radiation effects—and to establish an in vitro experimental system to facilitate these studies.

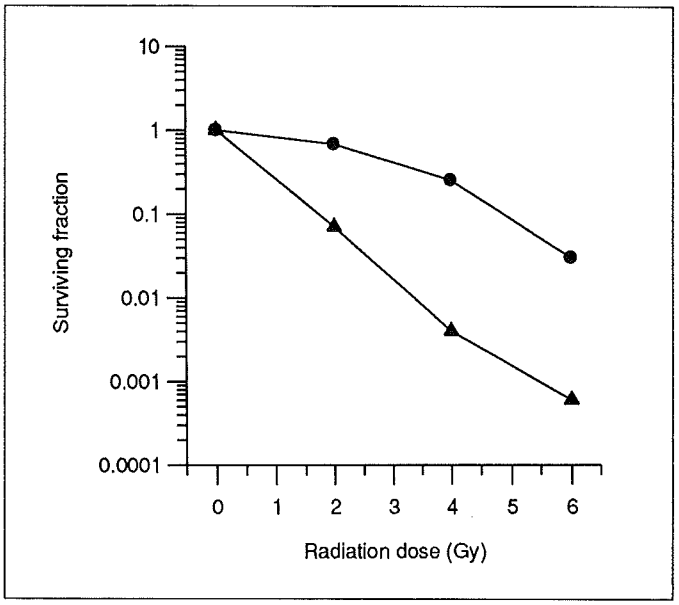


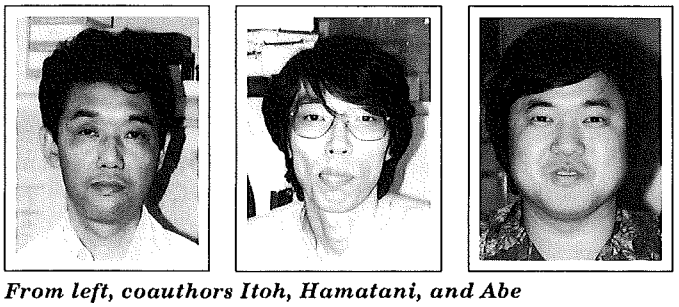
Figure 1. Radiosensitivity in SCID (▲) and human X SCID hybrid (●) cell lines.

Immunodeficiency and radiosensitivity: linked by a single gene in a mutant mouse

In 1983, M. J. Bosma and colleagues reported an animal model of severe combined immunodeficiency—a mouse lacking both T and B cells (GC Bosma et al, *Nature* 301:527–30, 1983). Subsequent analyses of this so-called SCID mouse revealed an abnormality in the rearrangement of immunoglobulin genes in B cells and of T-cell receptor genes in T cells, ie, a mutation of the V(D)J recombinase enzyme system that catalyzes these DNA rearrangement reactions. Because no mutant of this enzyme system has been isolated, the SCID mouse has become a very important tool for the study of this field (EA Hendricson et al, *Genes & Development* 2:817–29, 1988).

During our work using the SCID mouse, we noted in 1990 that a pre-B cell line established from the SCID mouse bone marrow is hypersensitive to radiation, as shown in Figure 1. About the same time, **G. M. Fulop** and **R. A. Phillips** reported that the SCID mouse is hypersensitive to radiation and that radiosensitivity in terms of cell survival is not restricted to lymphoid cells (GM Fulop and RA Phillips, *Nature* 347:479–82, 1990).

Furthermore, the results of a more detailed analysis performed by **A. B. Kim** et al suggested that the hyperradiosensitivity of the SCID mouse is an abnormality occurring during the repair of DNA double-strand breaks (AB Kim et al, *Proc Natl Acad Sci USA* 88:1394–7, 1991). Because the SCID mouse shows not only abnormal V(D)J recombination but also hyperradiosensitivity, it might be possible to clone a SCID mutant gene by selecting radiation-resistant cells derived from radiation-sensitive cells into which a normal gene, capable of restoring normal radiosensitivity, can be inserted. We, therefore, decided to analyze one mechanism that governs high radiosensitivity in humans by using the SCID mouse as an in vivo model.



From left, coauthors Itoh, Hamatani, and Abe

Defining a human chromosome that complements the radiosensitivity of SCID cells

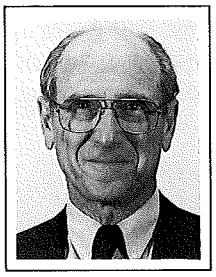
Our aim is to clone a SCID mutant gene. Before isolating such a mutant gene, we hoped to identify a human chromosome that complements the SCID mutation. To accomplish this, a SCID fibroblastic cell line was established from lung tissues of the C.B.17-*scid*/*scid* mouse by SV40 virus transfection. This cell line is highly sensitive to ionizing radiation. The hybrid cells obtained from fusion of the SCID fibroblastic cell line with normal human fibroblastic cells showed similar radioresistance to that observed in normal mouse fibroblastic cells.

To enrich the radioresistant cells, we eliminated the radiosensitive hybrid cells by repeated X-irradiations. Using this procedure, cells containing human chromosomes complementing the SCID mutation were allowed to survive. In the actual experiment, $5 \cdot 10^5$ cells were cultured

continued on next page

News Briefs

✓ Mendelsohn Assumes Post as Permanent Director



Mendelsohn

Mortimer L. Mendelsohn, former associate director for Biomedical and Environmental Research, Lawrence Livermore National Laboratory, began serving a 2-year term as one of RERF's six resident directors. Until his resignation in March, he had been cochairman of the Foundation's Scientific Council for 11 years.

✓ Results of 1991 Consultations with North American Atomic Bomb Survivors Available

From 11 June to 11 July 1991, 532 atomic bomb (A-bomb) survivors living in the United States and Canada met with Japanese doctors during the eighth cycle of consultations sponsored by the Hiroshima Prefectural Medical Association, the Japanese Ministry of Health and Welfare, Hiroshima prefecture and city, and the Hiroshima Atomic Bomb Casualty Council, in cooperation with several American medical associations.

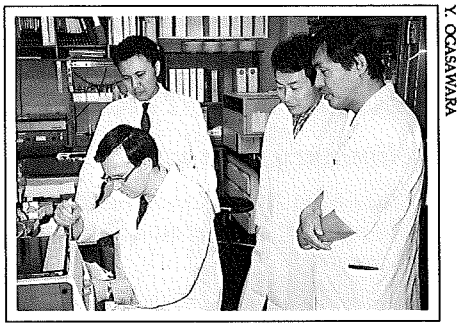
Of the confirmed 963 A-bomb survivors residing in North America, 149 men and 383 women were seen by the physicians—an examination rate of 50.1%. The mean age of participants was 61.2 years. Twenty male and 30 female offspring of A-bomb survivors also participated.

The results of the consultations showed that of the diseases requiring treatment and follow-up observation, hypertension presented the highest prevalence (27.6%), followed by hyperlipidemia, liver disease, thyroid disease, heart disease, and diabetes mellitus, in that order. An increase of liver and thyroid disease was observed. Two participants were invited to Japan for detailed examination and therapy, which led to the detection of colon cancer.

✓ Reprint Requests Received from 45 Countries

During fiscal year 1991–92, RERF received 653 reprint requests from 45 nations. Researchers from America requested 25% of the total. Investigators from Germany fol-

continued on page 11



Y. OGASAWARA

✓ Scientists from Russia and Kazakhstan Studied at RERF Hiroshima Laboratory in June

Scientists from regions of elevated radioactivity are frequent visitors to RERF. Above, seated, Alexander V. Akleyev (Ural Research Center of Radiation Medicine, Chelyabinsk, Russia) stains erythrocytes with anti-glycophorin A antibodies to measure somatic mutation frequencies, one step in biologically estimating radiation dose. Observing, from left, are Nurlan Shaimardanov (Medical Institute Semipalatinsk, Kazakhstan), RERF research scientist Seishi Kyoizumi and laboratory technician Kazumi Tanabe.

Gene Affecting Radiosensitivity

continued from page 5

in a 25-cm^2 flask. After incubation for 48 hours, the cells were X-irradiated (at 1 Gy) for 10 consecutive days. After an additional 10 days of growth culture, nine surviving colonies were randomly isolated. The colonies were cultured for 2–3 weeks and allowed to grow to a mass sufficient for selection after further X-irradiations, ie, three times at 3 Gy at 10-day intervals.

Although these radioresistant cell populations included a variety of human chromosomes in the initial stage, the cells containing human chromosome 8 became dominant. However, cells containing chromosome 16 or the X chromosomes also were present. After dispersing these cells in single cell culture, we obtained three radioresistant cell lines. Karyotype analysis revealed that these three cell lines contain human chromosome 8 (see Figure 2).

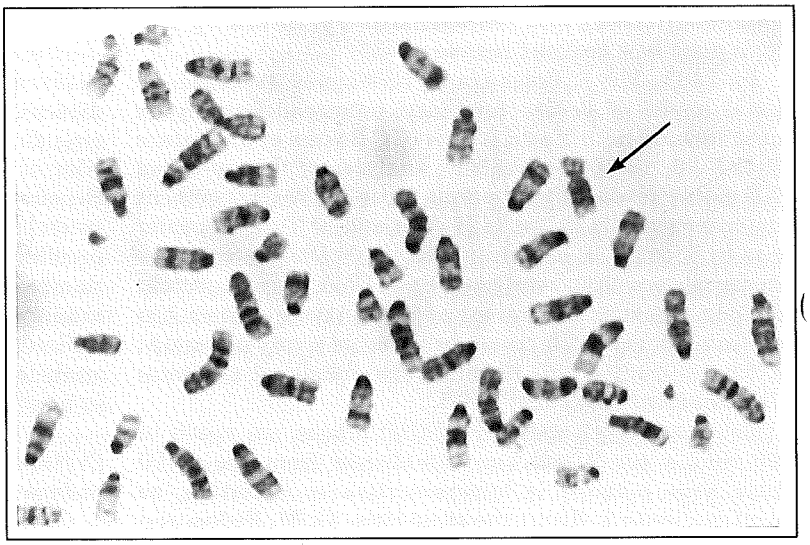


Figure 2. Metaphase chromosomes of the radioresistant cell line. The arrow indicates human chromosome 8.

In addition, to ascertain the relationship between human chromosome 8 and radiosensitivity, we established a cell line that had lost human chromosome 8 from the radioresistant cell line. Subsequently, we confirmed that this cell line's radiosensitivity was as elevated as the original SCID fibroblastic cell line.

These results strongly indicate that a gene capable of complementing the SCID mutation is located on human chromosome 8. Currently, we are fragmenting human chromosome 8 to determine experimentally the precise location of this gene, using the procedure described earlier. Simultaneously, we are attempting to isolate a gene responsible for the SCID mutation, using the DNA transfection technique.

Furthermore, our objective is to analyze the structure of the gene and the functions of its products. We hope that this analysis will eventually contribute to our understanding of one mechanism of radiosensitivity in humans. For this purpose, it is essential to perform further analysis of functions of individual components of this mechanism of radiosensitivity. The results to be obtained will enable us to establish a cell-free system to identify the gene products' biochemical function in causing immunodeficiency and radiosensitivity. ❑

Tumor Suppressor Genes: A Step Forward on the Road to Elucidating Human Carcinogenesis

The genetic alterations found in radiation-induced malignancies may offer clues to unraveling the processes of human carcinogenesis.

by Takashi Ito, Toshio Seyama, and Mitoshi Akiyama, RERF Department of Radiobiology, Hiroshima

For a malignant cancer cell to arise from a normal cell, multistep genetic alterations must take place over an extended time, with the number of steps varying according to the type of cancer. In this regard, one of the best characterized malignancies is colorectal cancer, which progresses from benign adenoma to carcinoma, and thus provides a unique system for the study of tumor progression. The genetic alterations occurring in this progression include oncogene activation (Ki-*ras*) at an early stage and loss or mutation of tumor suppressor genes at specific chromosomal loci including 5q (the *FAP* gene), 17p13 (the *p53* gene), and 18q21 (the *DCC* gene).

Oncogenes are generally thought to promote abnormal cellular proliferation by activation via amino acid substitutions, gene amplification, or gene rearrangements. Although many oncogenes, such as *ras*, have been identified, oncogenes alone do not supply an explanation for the mechanisms of carcinogenesis.

The crucial role of tumor suppressor genes

Tumor suppressor genes have only recently attracted much attention as playing a crucial role in negatively regulating cellular proliferation—their loss in activity through a deletion or a mutation can lead to uncontrolled cell growth. One of the most well-known tumor suppressor genes is the retinoblastoma gene (*Rb*), identified on chromosome 13. To explain the incidence of retinoblastoma, several years ago it was postulated that a "hit," or mutation, would have to occur in both alleles of the same gene on chromosome 13, thus predicting the recessive nature of this gene.

Recently, the retinoblastoma gene has been analyzed at the molecular level. The introduction and expression of the wild-type *Rb* gene in Rb^- cancer cells—including retinoblastoma, osteogenic sarcoma, and prostate carcinoma—effectively suppressed their tumor-forming ability. Additionally, cytogenetic and restriction-fragment length polymorphism (RFLP) analyses have provided initial mapping of putative tumor suppressor genes. Judging by the data collected to date, most of the genes in tumor cells that have lost heterozygosity turn out to be tumor suppressor genes. Using this form of analysis, a number of tumor suppressor genes now can be investigated.

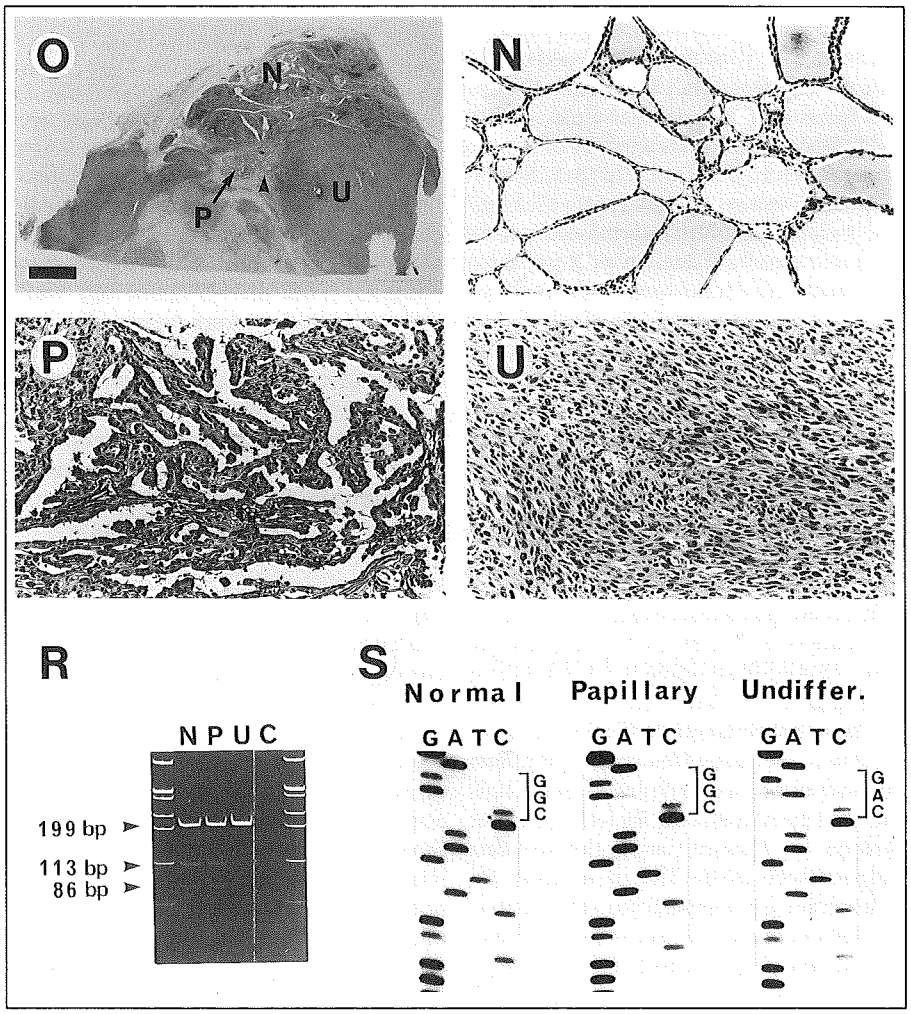


Figure 1. Histological findings and p53 gene analysis of undifferentiated carcinoma coexisting with papillary adenocarcinoma. Figure 1-O is an overview of the section, which contains normal thyroid tissue (N), papillary adenocarcinoma (P), and undifferentiated carcinoma (U). Polymerase chain reaction–restriction-fragment length polymorphism analysis of the p53 gene codon 72 (R) and polymerase chain reaction direct sequencing (S) using extracted DNA from each part showed allelic deletion of the p53 gene and a G → A transition at the second base of codon 248 exclusively at the foci of the undifferentiated carcinoma. The arrowhead (O) shows the PAC-undifferentiated carcinoma continuity. Scale bar: 0.5 cm. Original magnification: ×100 (N, P, U).

continued on next page

Tumor Suppressor Genes

continued from page 7

The *p53* gene is a well-studied tumor suppressor gene; its product is a 53-kD nuclear phosphoprotein, which was first discovered in SV40-transformed cell cultures. From transfection experiments, the *p53* gene was initially reported to be a dominant transforming oncogene. However, it is now understood that all *p53* genes having a cell-transforming ability are mutants and that the wild-type *p53* is able to suppress cell transformation. The human *p53* gene encompasses 20 kb of DNA on the short arm of chromosome 17 at position 17p13.1. Since human, monkey, mouse, rat, chicken, and frog *p53* cDNA has been cloned, comparison of all known *p53* peptide sequences has been possible, revealing that a large number of phylogenetically conserved amino acids are spread along the sequence. Moreover, five clusters of conserved positions have been noted. In the human sequence, they are localized between residues 13–19, 117–142, 171–181, 236–258, and 270–288. Most mutations that are detected among a diverse set of cancers cluster in these well-conserved domains.

The role of the *p53* gene in thyroid carcinogenesis

The normal function of *p53* has not yet been well described. Biochemical data have led researchers to hypothesize that *p53* exerts its function in the nucleus by forming a nucleoprotein complex with DNA. The idea that *p53* is involved in transcriptional regulation came from the sequence of the first 73 residues of the N-terminal domain that is highly acidic and similar to the acidic domains of many transcriptional factors. This domain appears to be able to activate transcription when fused to a DNA-binding polypeptide such as GAL4. In addition, *p53* has the ability to bind directly to DNA, probably by an interaction with its basic C-terminal region, which has a helix-coil-helix motif. Cotransfection experiments have demonstrated that transcription of the muscle-specific creatine kinase gene could be activated through *p53* binding. Furthermore, it has been suggested that wild-type *p53* acts as a transcriptional repressor of gene expression similar to interleukin-6. Future studies undoubtedly will clarify the functional role of *p53*.

At RERF, we are currently studying the role of *p53* mutations in thyroid carcinogenesis. Thyroid neoplasms show a variety of characteristics, ranging from slowly growing, well-differentiated tumors to rapidly progressive, highly malignant tumors. Pathological examination of coexisting tumors in the thyroid suggests that the undifferentiated carcinoma arises from differentiated tumors, mainly papillary adenocarcinoma (PAC) and follicular adenocarcinoma. Therefore, thyroid carcinoma may serve as an appropriate and interesting model for the investigation of multistep carcinogenesis. Point mutations in the dominantly acting activated *ras* oncogene—a candidate gene that is involved in the early stages of tumor progression—have been detected in benign tumors, well-differentiated carcinoma, and undifferentiated carcinomas. Rearrangements of two other candidate genes observed in PAC—*ret* and *trk*—also are believed to be involved in the early events of undifferentiated carcinoma development. Our recent studies indicate that *p53* mutations, which are associated with cell differentiation and the control of cell proliferation, are detected in undifferentiated carcinoma but not in well-differentiated carcinoma, as summarized in the table above.

Mutations of the p53 gene in thyroid carcinomas

Histological type[a]	Mutations in *p53* gene (positive/tested)[b]	Allelic loss (positive/informative)[c]
Papillary adenocarcinoma	0/10	0/4
Follicular adenocarcinoma	0/4	0/1
Undifferentiated carcinoma	7/8	3/5

[a]After microscopic identification, DNAs were extracted selectively from tumor tissues.

[b]Mutations in *p53* gene exons 5–8, determined by PCR-direct sequencing. All sequences were confirmed from forward and backward strands more than two times in order to exclude errors. All the positive cases showed base substitution mutations.

[c]Allelic loss in the *p53* gene, determined by PCR-RFLP at the BstUI site in exon 4, the MspI site in intron 6, and the ApaI site in intron 7. Using these three restriction sites, 4 out of 10 cases of PAC, 1 out of 4 cases of FAC, and 5 out of 8 cases of undifferentiated carcinoma were informative.

To confirm the involvement of the *p53* gene in the transition, mutations of *the p53* gene were examined using a coexisting tumor of undifferentiated carcinoma and well-differentiated carcinoma from the same individual. Examples of the pathological findings and the *p53* gene analysis are shown in the figure on the preceding page.

Figure 1-O shows a thyroid tissue section from a PAC (Figure 1-P) and undifferentiated carcinoma (Figure 1-U) foci surrounded by normal tissue (Figure 1-N). The arrowhead (O) indicates the transitional area between the PAC and the undifferentiated carcinoma. Polymerase chain reaction–restriction-fragment length polymorphism (PCR-RFLP) analysis revealed an allelic deletion in the undifferentiated focus but not in the differentiated (papillary) focus (Figure 1-R). Sequence analysis demonstrated a G → A transition at the second base of codon 248 in the undifferentiated carcinoma focus (Figure 1-S). These findings strongly indicated that *p53* mutations are involved in de-differentiation during tumor progression in the thyroid. Furthermore, the characteristically slow growth of well-differentiated carcinomas with no *p53* mutations and rapid growth of undifferentiated carcinomas with *p53* mutations imply that *p53* mutations that occurred during the growth of well-differentiated adenocarcinoma might result in undifferentiated carcinomas due to the effect they have on uncontrolled growth and de-differentiation.

Presently, we are attempting to elucidate the genetic alterations found in radiation-induced malignancies. The fact that ionizing radiation is more apt to cause deletion-type mutations rather than base-substitution–type mutations leads us to believe that tumor suppressor genes are appropriate targets of ionizing irradiation, resulting in radiation-induced carcinogenesis. We are now developing a method to detect deletions of the *p53* gene among cancers related to atomic bomb exposure.

Additional reading

M Holstein et al, *Science* 253:49–53, 1991

T Ito et al, *Cancer Res* 52:1369–71, 1992 ❑

Feedback

The Debate Continues: Threshold or No Threshold?

Bo Lindell, Statens strålskydds-institut, Stockholm, Sweden, writes:

I found the Perspectives editorial in the last issue (*RERF Update* 4(1):2, 1992) quite intriguing and hastened to read the articles by **V. P. Bond** and **James Trosko** (*RERF Update* 4(1):7–8; 3–5, 1992) to which you referred. At first when I read Bond's paper I did not appreciate the value that I assumed the paper must have, considering the author's reputation. However, when reading it, I became rather perplexed by the paper.

Bond starts from the postulation of a linear energy-response relation, illustrated in a diagram, relating the expected number of cancers in an exposed population to the collective energy ε in the "system," or to the collective dose in the population, which is the same thing.

On this assumption, there is a point where the expected outcome is one case of cancer. Here, Bond says, "...the curve is abruptly truncated." He continues: "This is because it is not possible to have a fraction of a cancer."

With the same reasoning, there cannot even exist a curve to be truncated, because it also is not possible to have 1.5 cancer or 3.87 cancers, etc. Bond obviously confuses observed events with mathematical expectations. His energy-response function (represented by the truncated straight line) is drawn as a best fit to the observed numbers of cancer at various system energies ε. The observed numbers must be whole numbers since 0.5 or 2.7 cases of cancer cannot be observed. But as soon as a best fit line is drawn, that line represents the mathematical expectation of the number of cancer cases at a given system energy. A mathematical expectation does not have to be a whole number (the mathematical expectation of the result from throwing a die is 3.5, although no die will ever show 3.5).

It is a mystery to me why Bond accepts a curve down to one cancer but not below one cancer. As an observed event, 1.5 cancer is as impossible as 0.5 cancer. As mathematical expectations, however, they are equally possible. A mathematical expectation of 0.1 cancer and a Poisson-distributed outcome give the observation of at least one case of cancer a probability of 9.5%. Bond's point of truncation (an expectation of one case of cancer) gives the observation of at least one case of cancer a probability of 63.2% (1 – 1/e). There is a difference in degree but not in principle. A probability of at least one case of cancer will exist at any value of ε, but will, of course, decrease as ε decreases. There is no discontinuity in the energy-response function. I would have thought this to be so obvious that I cannot understand why a reputable publication such as the *RERF Update* accepts such basic misunderstandings as those formulated by Bond.

It would have been acceptable if Bond had not so categorically talked about "threshold" and a "truncated curve" but had been content with suggesting that the system energy where the probability of *no* cancer is higher than 50% might be of some interest. This is the case when the mathematical expectation of the number of cancers is less than ln 2 = 0.693. With the energy-response relation that Bond assumed in his article, this corresponds to ε = 2 kJ or 35 man-Gy. With the risk estimate for *lethal* cancer in *ICRP Publication 60* (0.05 cases per man-Gy), however, and still assuming 60 kg per person, the value instead would be ε = 0.83 kJ or about 14 man-Gy, which, considering all uncertainties involved, would be better rounded off to ε = 1 kJ or approximately 10 man-Gy. The fact that, at lower system energies, it is more likely than not that no one will die from attributable cancer may have some value in risk communication.

The remaining individual probability of cancer, however, still might be unacceptable. For example, 1 man-Gy gives a mathematical expectation of 0.05 cases of cancer. With a Poisson distribution, the probability of having *some* death from attributable cancer at 1 man-Gy is still as high as $1 - e^{-0.05} = 5\%$. From the decisionmaker's point of view, this might be seen as a reassuring probability of 95% that there will be no attributable cancer death. However, if the size of the exposed population is merely 10 persons, they have an average individual dose of 100 mGy and hence an attributable cancer death probability of 1:200. Not shocking but hardly acceptable without very good reasons.

H. J. Dunster, Oxford, UK, also comments:

In *RERF Update* 4(1), V. P. Bond writes of an "energy threshold" for radiation-attributable cancer. His arguments appear to relate only to collective dose and do not address the primary question: "Is there a threshold dose to an individual below which there is no probability of attributable cancer?"

Bond's energy threshold of 3 kJ (about 43 person-Sv) is certainly a level at which attributable cancers cannot be demonstrated with statistical significance. With the high-dose projected lifetime probability coefficient derived from the Life Span Study (about 10% per sievert for fatal cancer), this collective dose is expected to produce only four attributable deaths. Even in a small exposed group of 40 persons, each with a dose of 1 Sv, the expectation number of attributable deaths is only four compared with the unexposed expectation of about 10. Even if the definition of significant is generously set at the 90% confidence limit, this increase is insignificant. In larger groups, the same absolute increase is even less significant.

But the problem of a probability threshold ought not be linked to the detectability of an effect. In a group of 40 people at an individual dose of 1 Sv, no risk can be detected. In the group of several thousand persons exposed at about this level in the Life Span Study, the excess number of cancers is clear. A subsample of 40 persons drawn at random from this group would not show a significant excess, but it would be absurd to suggest that the members of this subgroup were not at increased risk.

In *ICRP Publication 60*, the International Commission on Radiological Protection drew attention to a situation in which the radiation exposure of a group would have a high probability of causing no attributable cancers. It is necessary that the number in the group and the average dose should be small enough to make the expectation value of the attributable cancers much less than unity, say 0.01. At this expectation value, the probability of zero attributable cancers is 0.99, a level that can reasonably be called an effective threshold for this particular collective exposure. However, as the commission points out in paragraph 69, "...this provides no evidence for the existence of a real threshold."

If a threshold for individual risk

continued on next page

Threshold or Not?

continued from page 9

attributable to a radiation exposure is ever to be demonstrated or refuted, the basis for the conclusion cannot, in principle, be statistical. It will have to depend on a convincing mechanistic model for radiation carcinogenesis. That is not to decry the value of the "collective threshold" in making decisions in public health and in regulation. A collective dose in the region of 1 PSv will almost certainly cause no cancers. That is a useful piece of information, but it is not a threshold of individual risk.

Finally, I offer the suggestion that this may be simply a semantic issue. Does "threshold" mean a zero attributable risk or an attributable risk that is too small to be directly observed in a particular exposed group? To me, the latter meaning is an improper use of the word threshold.

V. P. Bond replies:

The criticisms in Bo Lindell's letter seem to hinge on two different but related statements in our summary article (*RERF Update* 4(1):7–8, 1992) which, when lifted out of context, are subject to widely disparate interpretations. One is that "it is not possible to have a fraction of a cancer"; the other that "the curve is abruptly truncated." With respect to the former, and noting the first, second, and fourth paragraphs of our article, I and my colleagues clearly meant that, because a fraction of a (person with) cancer cannot exist, it cannot be observed. Our article certainly was not intended to imply denial of the obvious fact that small values of ε can generate mathematical expectation values for (nonexistent) fractions of one cancer.

The meaning of the second quote is best enlarged on in the context of a fuller explanation of our approach, which, although provided in the reference articles, could not be included in the summary article. The key elements are as follows: A primary object of our approach was to obtain initially, from the atomic bomb survivor data, an empirically determined value for the average amount of energy deposited in the population that is required per observed (person with) cancer. This value, ε_0, could then be used, with any like and similarly exposed population for which ε is known, to predict the actual number of excess cancers to be expected. Our estimate for ε_0, 3 kJ, was also taken, without alteration, as a nominal value for a "threshold" (ie, the point on a function assumed to represent the data, below which the expectation value obtained from the function, for whatever reason, is no longer useful in approximating the number of responses actually observable in any practical case). That this value represents a conceptual threshold, which could be real only if the baseline ("normal") incidence were zero, is indicated by our calling this a minimal threshold that must, because of the large baseline cancer incidence, actually be much larger.

Our interest to date has been to establish principles and not to refine our nominal value of 3 kJ. Had we attempted this, adjustments would have been made for dose rate, excess cancers yet to appear among the survivors, the contribution of fast neutrons, the Poisson distribution, and the fact that we used only solid-tumor data and excluded those for leukemia. Without considering all of these potentially sizeable corrections, it would have been incongruous to adjust only for the (relatively small) Poisson factor on which Lindell focused his attention.

> ***'I and my colleagues clearly meant that, because a fraction of a (person with) cancer cannot exist, it cannot be observed. Our article certainly was not intended to imply denial of the obvious fact that small values of ε can generate mathematical expectation values for (nonexistent) fractions of one cancer.'***
>
> ***—V. P. Bond***

With respect to the abruptness of the truncation, our nominal value for ε_0, obtained from the one set of data we had to work with, is a single value. If a number of such sets had been available, obviously our value would be but one point on a distribution of such values. However, it is likely that the mean would be close to our nominal value of 3 kJ.

It is important that the value for ε_0, however obtained and however unrefined it may be, be marked on the function (eg, by truncation; a vertical dotted line; a dotted curve below this point). The reason is that, even though an observational threshold can exist on the usual dose-response curve, its location can be changed by altering the number of dosed subjects and thus ε. However, this is not true for the ε-response curve. In particular, the inclusion of more subjects below the threshold must inevitably increase ε at least to the value of ε_0, and usually much higher, before excess numbers can actually be observed. Thus, in principle and within the limits of error, ε_0 constitutes a conservative value for a stable and thus practical threshold or *de minimus* point, below which the expectation value will overestimate the excess number of responses that can actually be observed.

A principal conclusion from our overall approach, true whether or not adjustments are applied to our nominal value for ε_0 of 3 kJ, is as follows: the use of dose D as the independent variable to denote the "amount" of radiation energy is highly misleading because, being ambiguous with respect to mass, it applies equally from the smallest to the largest level of biological organization. Having an egocentric bent, people generally assume that the relevant mass must be that of one individual: hence the understandable and frightening (but unrealistic) interpretation that any amount of radiation to the individual, however small, can (and therefore probably will) result in an observable cancer. The use of ε instead of D forces the realization that the relevant "dose" is ε to the population and that if the required ε for one cancer were to be delivered to a "population" of one individual, the dose would be so large that death from early acute effects would render academic the question of a (delayed) cancer.

With regard to Dr. Dunster's letter, a key statement is: "But the problem of a probability threshold ought not be linked to the detectability of an effect." We agree with the statement, but not with the solution. As indicated in the last paragraph of our article, the time is perhaps overdue for us to cease dwelling on extrapolated individual probabilities that are of questionable value in population studies and we should focus instead on the much more meaningful attributable responses that are actually observable.

We are pleased to have the opportunity accorded us by virtue of Bo Lindell's and John Dunster's letters to expound additionally on our approach. ❑

Book Review

The Future of Human Radiation Research, edited by G. B. Gerber, D. M. Taylor, E. Cardis, and J. W. Thiessen, British Institute of Radiology, BIR Publications Office, 36 Portland Place, London W1N 4AT, UK, 1991, 174 pp.

What we *think* we know about human radiobiology is much less than what we are *sure* we do not know. This maxim is reaffirmed by the recent issuing of the proceedings of a unique workshop titled, "The Future of Human Radiation Research," that was held at Schloss Elmau, Germany, 4–8 March 1991. The gathering was sponsored by the US Department of Energy, RERF, the International Agency for Research on Cancer, and the Commission of the European Communities.

> ***'The message from this workshop was clear: our knowledge about human radiobiology is far from complete, and important new problems, especially in relation to low-level occupational exposures as well as to natural radiation sources, are still arising and need to be studied.'***
>
> ***—Proceedings editorial***

Because studies of the atomic bomb survivors will not be completed until well into the next century, the participants of this workshop brainstormed on how the information still to be gained might be increased by applying the latest technologies in epidemiology, statistics, and molecular biology.

Published by the British Institute of Radiology, the proceedings (BIR Report 22) outline the meeting's objectives: a) to evaluate the validity of the existing data on human radiobiology, b) to start new epidemiological and experimental approaches designed to reduce some uncertainties in the present data, and c) to examine the probable mechanisms of ionizing-radiation–induced human cancer.

Deviating from the usual workshop in format and variety of participant, the meeting stimulated a true dialogue among epidemiologists, statisticians, and risk-modelers, as well as a few radiobiologists and cancer specialists.

Data from studies of various human populations exposed to different types of radiation—including the atomic bombings, occupational exposures (uranium miners and radium dial painters), therapeutic medical exposures, and accidental exposures—were categorized and discussed according to organ systems. After each organized series of talks, sufficient time was allowed for the free exchange of ideas.

Compiled from prepared oral presentations, tape recordings, and participant notes, separate sections of the proceedings are devoted to reviews of data on specific forms of human cancer, eg, leukemia, cancer of the lung, breast, thyroid, or other sites, and of cancer induction for combined exposures to radiation and other carcinogens. The last two sections are dedicated to basic research approaches and analytical methodologies in statistics and epidemiology.

Risk assessment models, based only on individual empirical data without an understanding of the underlying biological bases, are in danger of being wrongly applied for policy and regulatory purposes. A recent article about the possible risks of mammography (M Allison, *Science* 256:1128–9, 1992) attests to the continuing need for new studies of human radiation exposure.

Statistical models being applied to various sets of epidemiological data, and findings and concepts from the field of chemical carcinogenesis, as well as the emerging field of "molecular epidemiology," were presented to the attending radiation epidemiologists. Gaps in knowledge were identified, new collaborations among various participants were initiated, and the need for a more biologically based risk assessment modeling system was expressed with renewed emphasis.

During the workshop, basic scientists, epidemiologists, and statisticians alike stressed their desire to carry out more mechanistic modeling—as opposed to simple mathematical modeling—of radiation-induced cancer risk. Opportunities for fruitful cross-discipline collaborations were recognized, although it was stressed that applying and interpreting mechanistic models should be carried out with caution: many models may fit equally well to the range of observed epidemiological data but predict very different risks outside this range. Participants generally agreed that additional research on the mechanisms specific to radiation carcinogenesis, as well as on the modes of interaction between radiation and other environmental agents in cancer induction, is needed to interpret correctly the results of epidemiological studies and to predict risks for other exposed populations.

—James T. Trosko

News Briefs

continued from page 6

lowed with 13%, France 10%, and the former Czechoslovakia 6%. Japan ranked 15th, requesting 2% of the reprints provided by RERF.

RERF reprints are available on request by contacting the RERF Publication and Documentation Center, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732 Japan.

✓ Research Staff News

Hiroshima

Department of Statistics: Research scientist **David J. Pawel** joined RERF in June. A mathematical statistician formerly employed by the US Food and Drug Administration, Pawel will provide statistical support for a variety of projects ongoing at RERF.

✓ Highlights of the RERF Lecture Program

Akihiro Shima, University of Tokyo, spoke on 1 May about the development of the Japanese medaka as a new model for studying environmental germ-cell mutagenesis.

On 15 May, **David C. Spray**, Yeshiva University, New York, NY, discussed the molecular physiology of gap junction channels.

On 1 June, **Fleming Brandt Sørensen**, Århus Kommunehospital, Århus, Denmark, lectured about the technical aspects and prognostic value of unbiased stereology used for objective histopathologic quantification in solid neoplasms.

On 8 July, **Colin R. Muirhead**, UK National Radiological Protection Board, spoke about mortality and occupational radiation exposure in the first analysis of the UK National Registry for Radiation Workers. ❑

'The Chrysanthemum and the Feather Merchant'

Excerpts from a 1961 presentation before the Chicago Literary Club offer yet another glimpse at the first National Research Council mission to Hiroshima and Nagasaki in 1946.

by Austin Moore Brues[†]

Editor's note: In earlier issues, James V. Neel (RERF Update 1[4]:7–8, 1989) and Paul Henshaw (RERF Update 3[4]:12–3, 1991) related their experiences during a National Research Council–sponsored trip in 1946–47 to report on prospects for setting up a research program in Hiroshima and Nagasaki. These selected recollections of Austin Brues, their colleague, have been culled from materials in the RERF archives.

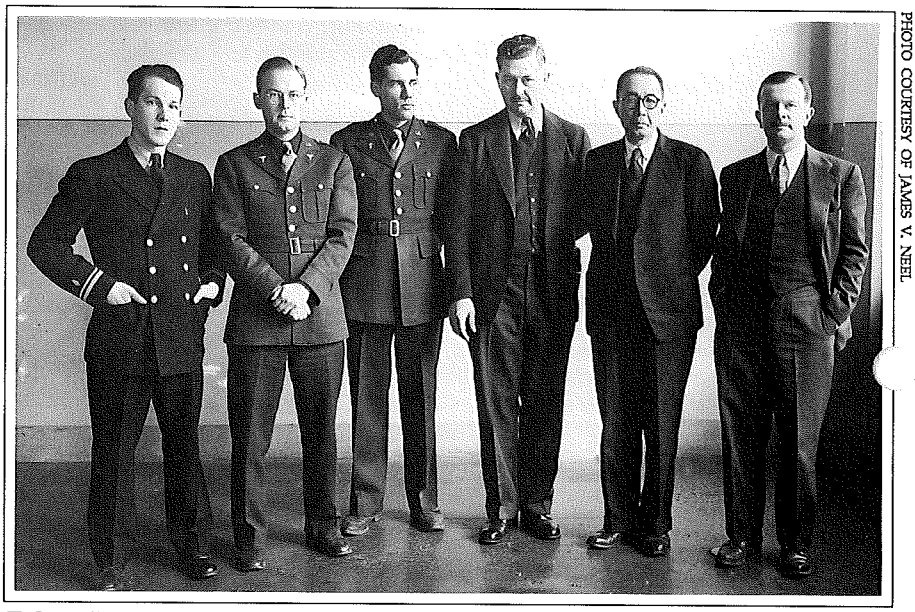


PHOTO COURTESY OF JAMES V. NEEL

Tokyo, January 1947. The first Atomic Bomb Casualty Commission consisted of the author, shown at far right, and his colleagues, from left, Frederick Ullrich, James Neel, Melvin Block, Paul Henshaw, and Masao Tsuzuki.

Arrival in Tokyo

First, we were taken to the Philippine Embassy, one of the available billets. This magnificent neo-Spanish colonial building turned out to be full of army cots, side by side. Fortunately, it was full, and we ended up at the Dai Iti [sic], or Number One Hotel. There, while little conferences were going on as to where, or whether, we would be placed, came the first lesson in conversational Japanese. The chap next to me asked me where I was from, Stateside, and I told him. When I asked him the like question, he said, "Ohio." Three Japanese maids walking by stopped at once, bowed formally, and said, "Ohayo," which means "Good morning."

At Tokyo Imperial University

One day we visited the Medical School of Tokyo Imperial University and called on **Masao Tsuzuki**, professor of surgery. After everyone had taken photographs of everyone else in front of a statue there, he took us to a large, dark, cold office piled to the ceiling with books. Frequently the telephone on the wall called him from his desk, and he would hold a long conversation.... It began to be clear who was organizing our expedition.

The Professor had spent a year or two in the States in the 1920s studying experimental radiology. He knew all of the physicians and most other people in Japan that counted for a great deal and had organized the first medical expedition to Hiroshima. In [a] most sensitive and vivid account [of that visit], *Hiroshima Diary*, Dr **Michihiko Hachiya** makes a point of the fact that Dr Tsuzuki's expedition, of course, did not have time to visit his rather humble hospital.

We were fortunate to have the Professor accompany us, since he knew his way around and in our spare time was a good teacher of the arts and culture. The good fortune was not all on one side, since he had been purged because he had done something for the Japanese Navy at a time when that constituted an un-American activity. This meant removal from his professorship, which was a government position, forcing him to private practice of surgery as a source of income. His participation in our activity was likely to maintain him in a good relation to the Occupation.

* * *

The hospital ward at the Imperial University, one of the distinguished medical centers, was swarming with people and pungent with the fishy odor of charcoal burners. Window glass was unobtainable even for hospitals.. Each patient had his meals cooked at the bedside by members of his family, often numerous and of all ages.

* * *

The next person to see was **Harry Kelly**, who was in charge of basic science for [the Occupation authorities]. Our own special concern with his office was in regard to arranging publication of a 100 or more papers on medical effects of atomic bomb radiation by the Japanese who had studied this firsthand.* A copy of a map of the bombed-out area in Hiroshima was guarded as security information in headquarters, while the rest of the edition was being sold at newsstands.

Japanese Professor Suspected Atomic Devices

I must delay the story of arriving in Hiroshima to tell the story of **Asada**, professor of physics at Osaka University, because it adds something to the official story. Shortly after the bombing of Hiroshima, Japanese physicists generally were quite sure the phenomenon could have been due to nothing other than a nuclear fission device. Asada was called

[†] *Austin Brues died at the age of 85 on 27 February 1991.*

**In 1953, "A Collection of Reports on Studies of A-bomb Casualties" was published in Japanese by the Science Promotion Society, Tokyo.*

before a general staff meeting in Tokyo and was offered anything he needed to build one in 6 months. He said, quite correctly, that it would be impossible to accomplish even in a year. A few days later he was again called to Tokyo and asked for a defense against these attacks. He said there was only one: to keep all planes from flying over Japanese territory. Within three hours of his answer, the surrender was announced. It would seem reasonable that the staff had received corroborating answers from other nuclear physicists.**

Kure and Hiroshima

Since the entire center of Hiroshima was missing there were not suitable quarters there, and the party was billeted in a compound in the hills near Kure, some 30 miles down the bay. Kure had an equal proportion of destruction, but it was dispersed and therefore less disruptive of communications. The buildings in the navy yard at Kure bore painted circles, squares, and triangles to mark their relative military importance for the guidance of the fire brigade. There must have been some good intelligence work, since those of the lowest priority were nearly intact, and those of the highest were practically all demolished. The navy yard was a beehive.

Hiroshima has been described in detail in many media before and after the official report of our mission. Most of our work there consisted of visiting the hospitals, and talking with physicians and midwives and with the public health authorities. A remarkably large proportion of the population bore large, thick, elevated keloid scars resulting from burns by the instantaneous heat of the explosion. These were so common that they were not accorded any attention except when they were so extensive as to be crippling.

The answer to the feasibility question seemed much more obvious in the bare center of the city than it had in the remote web of the Pentagon [where Brues received his first briefings on the mission]. In the first place, it would be absolutely inexcusable if a comprehensive study were not carried out, and already a year and a half had passed in which the unsupported Japanese had worked vigorously on their own and had hardly made an impression. In the second place, a properly conducted enterprise of this sort would be much more expensive than anybody Stateside could be induced to believe. If a great medical center could be brought to Hiroshima as a gift, the problem might be solved, but that sort of money was not now flowing as it had been when Peiping Union was endowed. What happened might have been expected: it got off to a slow start and appreciation of necessity and of the cost has developed slowly over many years.

Nagasaki

I wanted some relief from courtesy calls on minor politicians and clinic administrators, and Tsuzuki must have also, for when I suggested a visit to the area of radioactive fallout around the Nishiyama Reservoir, he quickly offered to come along. It was a day of relaxation, visiting farmer Nakao—who supplied soil, vegetables, and parts of a couple of rabbits and chickens—and collecting soil and water from the reservoir. We got a bit of almost everything that would characterize the fallout field except silt from the bottom of the reservoir. The project turned out of less value than it might have, because in a few years some better fallout fields were made; but it was a pleasant experience to see the hills in the tropical midwinter, and farmer Nakao, grateful for the honor of a visit to his humble place, gave me a pair of handmade straw slippers.

PHOTO COURTESY OF USNAS

The author in 1944

I remember especially two Nagasaki personalities. One was Furune, a horticulturist who was on a collecting trip in the mountains at the time of the bombing. His home was very close to ground zero and on his return he had to survey to find its former location. He reverently buried a few fragments of charred bone and built himself a small shelter of sheet metal until the Japanese press played up an apocryphal story by a press-happy New York physicist about the lethal qualities of the bomb field, after which [Furune] sought quarters in the city. Sixteen months later, the story was still given wide credence. While I talked with him at the still bare site of his home, three or four young press boys from a Nagasaki newspaper showed up with cameras and notebooks to get my opinion. I demonstrated that my watch was more radioactive than the ground.

* * *

But I have forgotten about Lieutenant [**Frederick**] **Ullrich** [another of the mission participants], who recovered from pink-eye in Tokyo while the major work of the expedition was going on. Since [he] felt very badly about having failed us in this uncomfortable and undramatic way, he was instructed to proceed to Nagasaki and procure the silt from the bottom of the Nishiyama Reservoir that would complete the fallout survey. About a month later, he arrived in Chicago rested from a sea voyage, bearing a sea chest containing, inter alia, a large can of mud.

Soon after that, [our] report was accepted, and the ABCC passed from the hands of some feather merchants—many of whom continue to advise and to work for it—to a duly constituted, permanent arm of the National Academy of Sciences. ❑

***Brues' colleague, Paul Henshaw, recalled the following in a manuscript written in 1991: "Dr Asada explained what he did on 6 August 1945, when he learned that a catastrophe had hit Hiroshima. At once, he said, he suspected an atomic device. He explained further that he gathered up a Geiger counter and an engineer's transet [sic] with small telescope and boarded a train going south. After traveling as far as he could toward Hiroshima, he turned on his Geiger counter and knew at once that an atomic bomb had been involved. Then by getting the angle indicated by burn shadows made by nails that had been driven part way into telephone poles and other similar arrangements at different locations, he quickly calculated the height at which the bomb was detonated. He considered his figures to be accurate within 5 feet, and they turned out to be within the 10-foot range estimated by the Americans and held as highly classified. Dr Asada, according to his story, did one other thing. He measured the radioactive decay rate of calcium and sulphur, respectively, in the bones of animals and in electrical insulators. With these figures, he calculated the amount of active materials detonated, but not the efficiency."*

Secular Changes of Population in the Adult Health Study

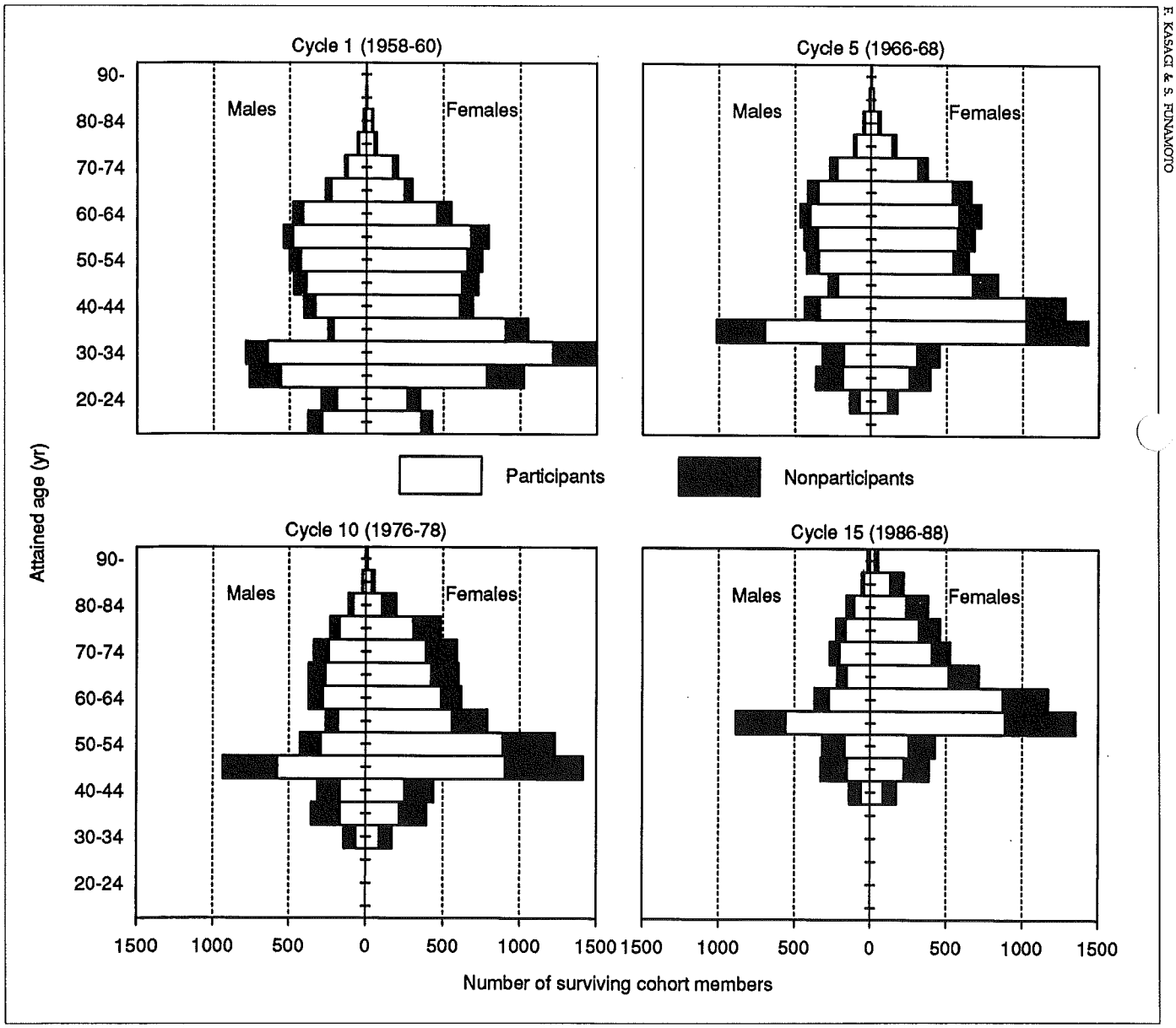
The Adult Health Study (AHS), a program of biennial medical examinations of a fixed subcohort of the ABCC-RERF Life Span Study (LSS) sample, was begun in 1958. The 18th cycle is currently underway. The procedure consists of medical history, physical examination, chest X ray, blood count, urinalysis, stool examination, electrocardiograms, and serum cholesterol determinations. Multiple blood biochemical measurements were initiated beginning in 1986 and ultrasonographic examination has been used since 1980 for diagnosing diseases related to abdominal organs.

The AHS cohort was originally defined as a fixed population of about 20,000 people. The cohort includes virtually all proximal survivors with acute symptoms of radiation exposure who were within 2000 m of the hypocenter and roughly equally sized age- and sex-frequency–matched samples of proximal survivors without acute symptoms, distal survivors, and nonexposed (not-in-city at the time of the bombings) controls. Since active follow-up of the nonexposed portion of the AHS ended in 1977, we focus on the exposed portion of the original AHS cohort known to have been alive in 1958—about 14,000 people.

These plots show the age and sex distribution of surviving members of the cohort for selected cycles. Persons refusing to undergo medical examination and migrants out of the contact area are shown as nonparticipants in the figure.

By the end of cycle 15 approximately 4900 deaths had occurred among members of the AHS. Although participation rates remain relatively high, there clearly has been a decrease in the size of the cohort and, conversely, an increase in the proportion of surviving cohort members over 80 years old. ❑

Erratum: The subject matter of the last Facts & Figures column (*RERF Update* 4[1]:13, 1992) was excess relative risk rather than estimated relative risk as indicated in the text and title.



F. KASAGI & S. FUNAMOTO

Recent Scientific Publications

Editor's note: The reports listed have been approved and will be distributed as soon as they are printed. Wording of the titles and summaries may be slightly altered before final printing.

Approved Technical Reports

Unique Association of *p53* Mutations with Undifferentiated but Not with Differentiated Thyroid Gland Carcinomas. T Ito, T Seyama, T Mizuno, N Tsuyama, T Hayashi, Y Hayashi, K Dohi, N Nakamura, M Akiyama. **RERF TR 3-92.**

Thyroid neoplasms show a wide variety of lesions ranging from slowly growing differentiated adenocarcinomas to rapidly proliferating undifferentiated carcinomas. There is some histopathological evidence that the undifferentiated thyroid carcinomas are derived from differentiated carcinomas. Moreover, it is suspected that some genetic events might be associated with such changes. In the present study, mutations in the *p53* gene were detected by direct sequencing analysis after polymerase chain reaction amplification of exons 5 to 8, using paraffin-embedded primary tumors and cultured cells.

No mutations in exons 5 to 8 were detected in 10 differentiated papillary adenocarcinomas, whereas 6 out of 7 undifferentiated carcinomas were found to carry base substitution mutations. Sequencing analysis confirmed mutations at codons 135 (TGC → TGT), 141 (CCC → CCT), 178 (CAC → GAC), 213 (CGA → TGA), 248 (CGG → CAG, CGG → TGG), and 273 (CGT → TGT). The spectrum of mutations (G:C to A:T transitions in 7 of 8) might be a specific feature of the spontaneous cancers. The results strongly suggest that, in human thyroid glands, *p53* mutations play a crucial role in the progression of differentiated carcinomas to undifferentiated ones.

Autoantibodies and Immunoglobulins among Atomic Bomb Survivors. S Fujiwara, RL Carter, M Akiyama, M Akahoshi, H Sasaki, M Yamada, K Neriishi, S Kusumi, K Kodama, K Shimaoka. **RERF TR 4-92.**

The purpose of this study was to determine if exposure to atomic bomb radiation affects immune responsiveness, such as the occurrence of autoantibodies and levels of immunoglobulins. Rheumatoid factor, antinuclear antibody, antithyroglobulin antibody, anti-thyroid-microsomal antibody, and immunoglobulin levels (IgG, IgM, IgA, and IgE) were measured among 2061 Adult Health Study participants in Hiroshima and Nagasaki from December 1987 to the end of November 1989.

The prevalence and titers of rheumatoid factor increased in a statistically significant manner as radiation dose increased. No radiation effect was found on the prevalence of antinuclear antibody, antithyroglobulin antibody, and anti-thyroid-microsomal antibody.

A statistically significant relationship also was found between the IgA level in females and the IgM levels in both sexes—they increased as radiation dose increased. However, the effects of radiation exposure were not great and accounted for less than 10% of the total variation in each measurement. Levels of IgG and E were not affected by radiation exposure.

Solid Tumor Incidence in Atomic Bomb Survivors, 1958–87. D Thompson, K Mabuchi, E Ron, M Soda, M Tokunaga, S Ochikubo, S Sugimoto, T Ikeda, M Terasaki, S Izumi, D Preston. **RERF TR 5-92.**

This report presents, for the first time, comprehensive solid cancer incidence data and risk estimates for A-bomb survivors in the extended Life Span Study (LSS-E85) cohort. Among 79,972 individuals, 8613 first primary solid cancers were diagnosed between 1958 and 1987. As part of the Hiroshima and Nagasaki tumor registries' standard registration process, cancer cases occurring among members of the LSS-E85 cohort were identified using a computer linkage system supplemented by manual searches. Special efforts were made to ensure complete case ascertainment, data quality, and data consistency in the two cities. For all sites combined, 75% of the cancers were verified microscopically, 6% were diagnosed by direct visualization, 8% were based on a clinical diagnosis, and 12.6% were ascertained by death certificate only.

A standard set of analyses was carried out for each of the organs and organ systems considered. Depending on the cancer site, DS86 organ or kerma doses were used for computing risk estimates. Analyses were based on a general excess relative risk model (the background rate multiplied by 1 plus the excess relative risk). Analyses carried out for each site involved fitting the background model with no dose effect, a linear dose-response model with no effect-modifiers, a linear-quadratic dose-response model with no effect modifiers, and a series of linear dose-response models that included each of the covariates (sex, age at exposure, time since exposure, attained age, and city) individually as effect modifiers. Because the tumor registries ascertain cancers in the registry catchment areas only, an adjustment was made for the effects of migration.

Consistent with prior LSS findings on mortality, a statistically significant excess risk for all solid cancers was demonstrated (excess relative risk at 1 sievert [ERR_{1Sv}] = 0.63; excess absolute risk [EAR] per 10^4 person-year-sievert [PYSv] = 29.7). For cancers of the stomach (ERR_{1Sv} = 0.32), colon (ERR_{1Sv} = 0.72), lung (ERR_{1Sv} = 0.95), breast (ERR_{1Sv} = 1.59), ovary (ERR_{1Sv} = 0.99), urinary bladder (ERR_{1Sv} = 1.02), and thyroid (ERR_{1Sv} = 1.15) significant radiation associations were observed. There was some indication that there was an increase of tumors of the neural tissue (excluding the brain) among persons exposed to the bombings before age 20. For the first time, radiation has been associated with liver (ERR_{1Sv} = 0.45) and nonmelanoma skin (ERR_{1Sv} = 1.0) cancer incidence in the LSS cohort. The present analysis also strengthened earlier findings, based on a smaller number of cases, of an effect of A-bomb radiation on salivary gland cancer. There was no significant radiation effect for cancers of the oral cavity and pharynx as a group, or of the esophagus, rectum, gallbladder, pancreas, larynx, uterine cervix, uterine corpus, prostate, kidney, and renal pelvis.

Analyses of solid tumors individually and in combination revealed no appreciable differences between Hiroshima and Nagasaki ($p > 0.5$). The combined solid tumor analysis demonstrated a twofold greater relative risk for females than males and a trend for a decreasing relative risk with increased age at exposure ($p < 0.001$). Females had a higher relative risk of cancers of the lung, total respiratory system, and urinary system than males. The excess relative risk decreased with increasing age at exposure for salivary gland, combined gastrointestinal, stomach, skin, breast, and thyroid cancers. For solid cancers combined, the excess cancer rate increased with increasing attained age and was proportional to the background incidence rate. Unadjusted for age at exposure, the excess relative risk for most sites tended to decrease with increasing attained age. For some cancers (colon, breast, central nervous system, and kidney), models that allowed the excess relative risk to vary with attained age fit at least as well as models that included age-at-exposure effects. For all solid tumors, excess cancers increased with time since exposure based on an absolute excess risk model. Averaged over all ages at exposure, the relative risk decreased with time since exposure. Examination of temporal patterns by age-at-exposure groups suggested that the excess relative risk decreased with time for the younger age-at-exposure groups and remained virtually constant for the older cohorts.

The LSS has served as one of the major sources of data used for cancer risk estimation. Previous studies focused primarily on the association between cancer mortality and radiation exposure. Although these mortality studies are extremely valuable, the accuracy of cancer diagnoses is limited, and death certificates do not provide adequate information on cancers with relatively high survival rates. Although incidence data also have their limitations (eg, completeness of case ascertainment and partial reliance

continued on next page

Recent Scientific Publications

on death certificate diagnoses), they can provide more complete data on the less lethal cancers, histologic type, and on time from exposure to cancer onset. Thus, future analyses of A-bomb survivors should focus on both cancer mortality and incidence.

Approved Research Protocols

Tumor Suppressor Gene Alterations in Lung Tumors from Japanese Mustard Gas Workers and Atomic Bomb Survivors. Y Takeshima, T Seyama, WP Bennett, RA Metcalf, S Akiba, M Fujihara, Y Hayashi, S Yonehara, T Ito, T Mizuno, K Inai, M Yamakido, N Nakamura, M Akiyama, S Tokuoka, K Mabuchi, CE Land, CC Harris. **RERF RP 3-92.**

The proposed study will investigate alterations of the *p53* tumor suppressor gene and protein within archival lung tumor samples from Japanese mustard gas factory workers and atomic bomb survivors. Although the ultimate objective of this comparative study is to determine whether the presumptive "carcinogens," namely, mustard gas and ionizing radiation, leave unique "molecular fingerprints" in specific genes involved in the carcinogenic process, many limitations will probably preclude any definitive conclusions. These limitations include small sample size, that is, scarcity of available material, and the inability to detect deletion mutations at this gene locus.

Therefore, the study is intended as a pilot study to a) develop expertise in this area for future studies in "molecular epidemiology," b) identify the potentials and limitations of these kinds of studies for prospective RERF initiatives in this area, c) contribute to a larger series of studies being conducted on the *p53* target gene in tumors of humans exposed to other known carcinogens, and d) help contribute to the development of new technologies to detect deletion mutations involving this *p53* tumor suppressor gene.

Incidence Study of Tumors of the Central Nervous System among Atomic Bomb Survivors. S Yonehara, H Fujii, M Kishikawa, T Kobuke, S Fujita, M Soda, M Tokunaga, S Tokuoka, D L Preston, K Mabuchi, E Ron, CE Land. **RERF RP 4-92.**

Few reports have been published on the risk of tumors of the central nervous system due to radiation exposure. The proposed study will assess, under the RERF guidelines for the conduct of site-specific cancer incidence studies, tumors of the central nervous system in the period 1950–87 among male and female members of the RERF extended Life Span Study cohort in Hiroshima and Nagasaki. The shape of the dose-response curve and the risk by city, age at the time of the bombings, time since exposure, and type of tumor will be examined.

Tumor cases will be ascertained mainly from autopsy records, surgical pathology records, and death certificates maintained at RERF as well as from the tumor and tissue registries of Hiroshima and Nagasaki. Consideration also will be given to the detection and collection of cases from clinical records as well as autopsy and surgical pathology records maintained at major medical institutes in both cities.

Study on Senile Dementia among the Adult Health Study Subjects in Hiroshima and Nagasaki. H Sasaki, K Oishi, M Yamada, K Kodama, M Akahoshi, K Shimaoka, S Nakamura, M Tsujihata, S Nagataki, M Seto, F Kasagi, Y Shimizu, Y Shibata, JL Ohara, LR White, WJ Schull. **RERF RP 5-92.**

A wide spectrum of radiation effects on the central nervous system has been well documented in clinical and epidemiologic studies, especially for individuals who were exposed prenatally, or postnatally, in particular during childhood. Nonetheless, the hypothesis that exposure to ionizing radiation accelerates the aging process has been actively investigated for a number of years at ABCC-RERF; however, the results have not been consistent. The effects of ionizing radiation on the mature central nervous system could possibly be manifested as an accelerated neurologic aging, but this has not been well established yet.

The study proposed here will examine the association, if any, between exposure to the atomic bombings and the subsequent impairment of cognitive function and the occurrence of senile dementia. To ascertain demented persons, standardized screening instruments for cognitive function, history of cognitive deterioration, and so on will be used. In the clinical evaluation of patients with dementia, standardized neurological examinations will be performed, including CT scan and magnetic resonance imaging. We also will attempt to determine those factors, if any, that modify the association of these neurologic disorders with ionizing radiation.

Approved Commentary and Review

Application of Generalized Estimating Equations to a Study of In Vitro Radiation Sensitivity. JB Cologne, RL Carter, S Fujita, S Ban. **RERF CR 2-92.**

We describe an application of the generalized estimating equation (GEE) methodology (Liang and Zeger, 1986; Zeger and Liang, 1986) for regression analysis of correlated Poisson data. As an alternative to the use of an arbitrarily chosen working correlation matrix, we demonstrate the use of GEE with a reasonable model for the true covariance structure among repeated observations within individuals. We also illustrate the use of GEE with an empirically estimated model for overdispersion. We conclude by summarizing issues and needs for further work concerning efficiency of the GEE parameter estimates in practice.

Publications in the Open Literature

Evaluation of Cancer Prevention Strategies by Computerized Simulation Model: an Approach to Lung Cancer. N Yamaguchi, Y Tamura, T Sobue, S Akiba, M Ohtaki, Y Baba, S Mizuno, S Watanabe. *Cancer Causes and Control* 2:147–55, 1991.

Unique Association of p53 Mutations with Undifferentiated but Not with Differentiated Carcinomas of the Thyroid Gland. T Ito, T Seyama, T Mizuno, N Tsuyama, T Hayashi, Y Hayashi, K Dohi, N Nakamura, M Akiyama. *Cancer Res* 52:1369–71, 1992. (RERF TR 3-92) ❑

RERF **update** RERF

This quarterly newsletter is published by the Radiation Effects Research Foundation (formerly the Atomic Bomb Casualty Commission), established in April 1975 as a private, nonprofit Japanese foundation. It is supported equally by the Government of Japan through the Ministry of Health and Welfare and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.

RERF conducts research and studies—for peaceful purposes—on the medical effects of radiation on humans with a view toward contributing to the maintenance of the health and welfare of atomic bomb survivors and to the enhancement of the health of all mankind.

Editorial Policy
Contributions to *Update* receive editorial review only and are not subjected to scientific peer review. Consequently, the opinions expressed herein are those of the authors only and do not necessarily reflect RERF policies or positions.

Units of radiation and radioactivity are given as found in the source material.

Editorial Staff
Editor-in-chief: J. W. Thiessen
Managing editor: Beth Magura
Assistant editor: Jeanette Nakada
Production asst: Fumie Maruyama
Photographer: Junso Takayama

Mailing Address
RERF Update
5-2 Hijiyama Park
Minami-ku, Hiroshima
732 Japan