RERF update RERF

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

Volume 2, Issue 4 **Hiroshima & Nagasaki** **Winter 1990–91**

Collaborative Radiation Research Center Planned

The misfortunes of war—and more recently of industry—are the ironic cornerstones in an unprecedented international attempt to mobilize the modern world's financial, administrative, and scientific resources on behalf of the millions of people involved in the Chernobyl nuclear power plant accident of April 1986.

Meeting in Hiroshima for the first time, the World Health Organization's Scientific Advisory Committee convened 23–26 October to lay the groundwork for establishing the International Center for Radiation Health Issues, an organization that will be devoted to systematically studying the aftermath of the Chernobyl accident.

The center will be located in Obninsk, a city near Moscow, with branch laboratories in Kiev, the Republic of the Ukraine, as well as in Brynsk, the Republic of Russia, and Gomel, the Republic of Byelorussia.

"In light of the many changes occurring in the Soviet Union at this time, the launching of the radiation health center will depend on international staffing and training, and financial assistance from abroad," commented RERF Chairman **Itsuzo Shigematsu**, who is a member of the WHO advisory committee.

"The experience to be gained in minimizing the health consequences of such a large-scale accident can be of value to other countries, as well as to the USSR," continued Shigematsu, who also chairs an IAEA committee charged with evaluating the impact of the reactor explosion.

J. TAKAYAMA

At the city museum in Peace Memorial Park, participants of the WHO Scientific Advisory Committee meeting view a diorama showing the widespread destruction of Hiroshima City resulting from the atomic bombing. From left, P.J. Waight, F.A. Mettler, T.R. Lee, L.A. Ilyin, A.F. Tsyb, Museum Director Y. Kawamoto, N.P. Bochkov, Wu De-Chang, S. Nagataki, and two bystanders. Representatives from 13 nations gathered in Hiroshima to draft specific recommendations for establishing the International Center for Radiation Health Issues, which will be located near Moscow.

The four-day WHO Scientific Advisory Committee meeting produced six recommendations that call for:

- an epidemiological study of the exposed residents, emergency accident workers, and others from the affected regions,
- estimation of the radiation doses received by the population,
- mitigation of the sociopsychological effects on residents of the affected areas,
- clinical follow-up of the residents,
- education and training of a research staff, and
- design and maintenance of a health information data base. ❑

Meeting participants

F. Bégon, Service de Médecine Nucléaire, France
D. Beninson, Comision Nacional de Energia Atomica, Argentina
N.P. Bochkov, USSR Medical Academy (chairman)
A.M. Kellerer, Institute for Radiation Biology, Germany
T.R. Lee, University of St. Andrews, UK
K.H. Lokan, Australian Radiation Laboratory
F.A. Mettler, University of New Mexico School of Medicine, USA
I. Shigematsu, RERF, Japan
Wu De-Chang, Institute of Radiation Medicine, People's Republic of China

WHO Secretariat:
I. Riaboukhine
P.J. Waight

Representatives of international organizations:
M. Rosen, International Atomic Energy Agency, Vienna
F. Luykx, Commission of the European Communities, Luxembourg
G. Gerber, Commission of the European Communities, Brussels

Representatives of the USSR:
L.A. Ilyin, Ministry of Health
A.F. Tsyb, Research Institute of Medical Radiology

Observers:
Y. Skoropad, Research Institute of Medical Radiology, USSR
A. Kuramoto, Hiroshima University
S. Nagataki, Nagasaki University School of Medicine
I. Honma, Japanese Ministry of Health and Welfare
T. Toguchi, Japanese Ministry of Health and Welfare
Y. Aoki, Japanese Science and Technology Agency
J.W. Thiessen, RERF, Japan
Y. Hasegawa, RERF, Japan ❑

Perspectives

Atomic Bomb Survivors and Low-level Radiation

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

In an earlier column (*RERF Update* 1(3):2, 1989), I pointed out that of all A-bomb survivors only a relatively small fraction had actually received radiation doses greater than 0.1 Gy, viz., the so-called proximal survivors. Only the nearest of the distal survivors received doses higher than 0.01 Gy. As can be seen in the Facts and Figures column on page 8, of the survivors covered in the Life Span Study (LSS) for whom DS86 kerma estimates are available, about 24,000 have been assigned values between 0.01 and 0.1 Gy, and nearly 13,000 have a kerma between 0.1 and 0.5 Gy. Only a little over 7,200 survivors have been assigned kerma values greater than 0.5 Gy.

Two points can be made. First, the RERF data base is important not only with respect to high-dose data. It is also of more than cursory interest on the subject of "low-level radiation" although this is not generally realized, as evidenced by the most recent comprehensive reports by UNSCEAR and the BEIR Committee, which make no mention of this fact. Second, the great majority of excess cancers occurs in those with a kerma greater than 0.5 Gy, i.e., in a relatively small fraction of the exposed survivors. This greatly affects the way the risk of low-level radiation exposure is evaluated. I wonder how we would look upon our data if no survivor had received any exposure resulting in kerma greater than 0.5 Gy.

All this may seem to be of marginal importance, with "everyone" agreeing on risk estimates, risk projection models, etc. I, for one, am not so sure that the famous linear-quadratic dose-response assumption, with no threshold, is as cast in concrete as one is made to believe, e.g., in the new ICRP recommendations soon to be published. What once was considered to be a cautious assumption necessary for the protection of health—a consideration I cannot argue with (at least not yet)—is developing into a statement of scientific fact, or worse, an article of faith.

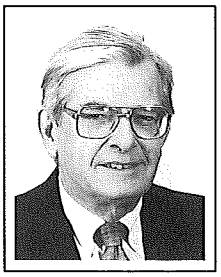
I find this rather curious at a time when serious doubt is being cast on the correctness of carcinogenesis models entirely based on mutagenesis as the determining factor (see, as two of an increasing number of examples, **B. Ames**, *Science* 249:970–971, 1990, and *Proc Natl Acad Sci USA*, 87:7772–7776, 1990). Many scientists are now beginning to believe that cell killing with the concomitant activation of cell-growth factors and deactivation of growth inhibitors may play an essential role in carcinogenesis. If that is so, then low-level radiation, with its absence of a cell-killing effect, may well demonstrate a threshold, a point made, in a slightly different context, by RERF Chief of Research **James Trosko** (*Radiat Res* 123:241–251, 1990, and in *Biologically based methods for cancer risk assessment*, edited by C.C. Travis, New York, Plenum Press, 1989, pp. 165–179).

Of course, it is doubtful whether our epidemiologic data will ever be able to demonstrate, with an adequate degree of confidence, the existence (or absence) of a real threshold at low doses, notwithstanding the apparent threshold in the LSS data somewhere between 0.2 and 0.5 Gy (Shimizu et al., RERF TR 5-88). Even though more than half of the survivors in the LSS are still alive and therefore a substantial number of cancers is yet to occur, statistical power may never be adequate for that to happen. However, an ever growing data set in the tumor registries is also a source of information that we are now beginning to evaluate (see *RERF Update* 2(2):5–6, 1990). Maybe these data are (or will become) amenable to a more powerful analysis in the low-dose range. In any case, it appears to me that the resolution of critical questions about the shape of the response curve at low doses is much more likely to be found in the RERF data sets than in the jumble of studies on radiation workers and populations around nuclear facilities or exposed to fallout that are usually referred to when low-level epidemiologic studies are discussed.

In the end, resolution may depend on better insights into the mechanisms of carcinogenesis in general and radiation carcinogenesis in particular. It is appropriate that RERF carefully analyze its abilities to help in providing such insight, applying the advanced research methodology now available and using the tissue, cell, and sera specimens obtained from survivors in the past. Such analyses are now underway. ❑

News Briefs

✓ Schull Returns to RERF as Permanent Director



Schull

Since mid-November, **William J. Schull** has been serving as one of RERF's six permanent directors. His association with ABCC–RERF dates back to 1949, when he first came to Japan to head the genetics program at ABCC. Schull previously served as RERF vice chairman from 1978–80, then again as a permanent director from 1986–87. He is on leave from the Genetic Centers of the Graduate School of Biomedical Sciences, University of Texas Health Science Center in Houston, where he is director.

(See *RERF Update* 2(3):6–9, 1990, for articles on the early years of the ABCC genetics program, and *RERF Update* 2(2):8, 1990, for a review of Schull's book, *Song among the ruins*, which describes his early experiences at ABCC–RERF and in Japan.)

✓ US Science Representatives Visit RERF Laboratories

The Hiroshima and Nagasaki labs were recently visited by **Edward M. Malloy** and **Evans J.R. Revere** of the US Embassy and **Robert B. Hardy** and **Charles W. Wallace** of the National Science Foundation. Since RERF is now eligible to receive foreign scientists under the Japanese Science and Technology Agency's fellowship program, RERF directors and department chiefs provided an overview of the research program for Hardy and Wallace.

✓ Citizens' Group Invites Soviet Scientists to Study in Hiroshima

In November, RERF, Hiroshima University's Research Institute for Nuclear Medicine and Biology, and other local radiation research institutions hosted two Soviet physicians from Semipalatinsk, Republic of Kazakhstan, where nuclear testing has been conducted for more than 40 years. Invited to Hiroshima by a citizens' group, **Boris Gusev**, Semipalatinsk Research Institute for Radiation Medicine, and **E.A. Baysembaev**, Pavlodar Clinical Immunology Center, brought medical data on long-term, low-dose exposures. They conferred with the governor of Hiroshima Prefecture, **Toranosuke Takeshita**, who expressed the prefecture's willingness to receive Soviet specialists for longer term training.

✓ In Memoriam: Yoneta Ichikawa and Councilman Morgan

Yoneta Ichikawa, a contributor to the two-volume report *US–Japan joint reassessment of atomic bomb radiation dosimetry in Hiroshima and Nagasaki*, died on 29 August of prostatic cancer at the age of 65. Formerly professor emeritus at Nara University of Education, his specialty was basic physics.

Councilman Morgan, an RERF visiting director from 1977–79, died on 10 October at the age of 70 as a result of complications after open-heart surgery.

✓ A Sweet Dosimeter?

Researchers at the Japanese National Institute of Radiological Sciences reported measuring average doses of 0.055 Gy and

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The Future of the Biochemical Genetics Study

The author discusses new approaches that could be used in the '90s for further genetic evaluation of the children of atomic bomb survivors.

by Chiyoko Satoh
Laboratory of Biochemical Genetics

Follow-up studies on the potential genetic effects of the atomic bombs detonated over Hiroshima and Nagasaki were initiated in 1946 and have continued to the present time. Extensive studies on the children of survivors have thus far yielded no evidence of any statistically significant increase in genetic effects when compared to a control population.

With particular reference to biochemical studies, 11,364 children of exposed parents whose average combined gonadal dose was ≥0.01 Sv and 12,297 control children whose parents' gonadal dose was <0.01 Sv were screened for de novo mutations of 30 blood proteins, detected by their altered ability to migrate in a gel matrix under the influence of an electric field (one-dimensional gel electrophoresis). Two and four mutations resulting in altered mobility were detected in 544,779 and 589,506 equivalent gene tests for the former and the latter groups, respectively. (Because of the new dose estimation, numbers of mutations and gene tests in the two groups differ somewhat from those reported by Neel et al., *Am J Hum Genet* 42:663–676, 1988.) The mutation rate per gene per generation for the exposed group was $0.37 \cdot 10^{-5}$ and that for the control group $0.68 \cdot 10^{-5}$. A subset of the children, 4,989 of the former and 5,026 of the latter, was further screened for loss of activity in nine erythrocyte enzymes. One mutation was detected in the exposed group, whereas none was detected in the control group.

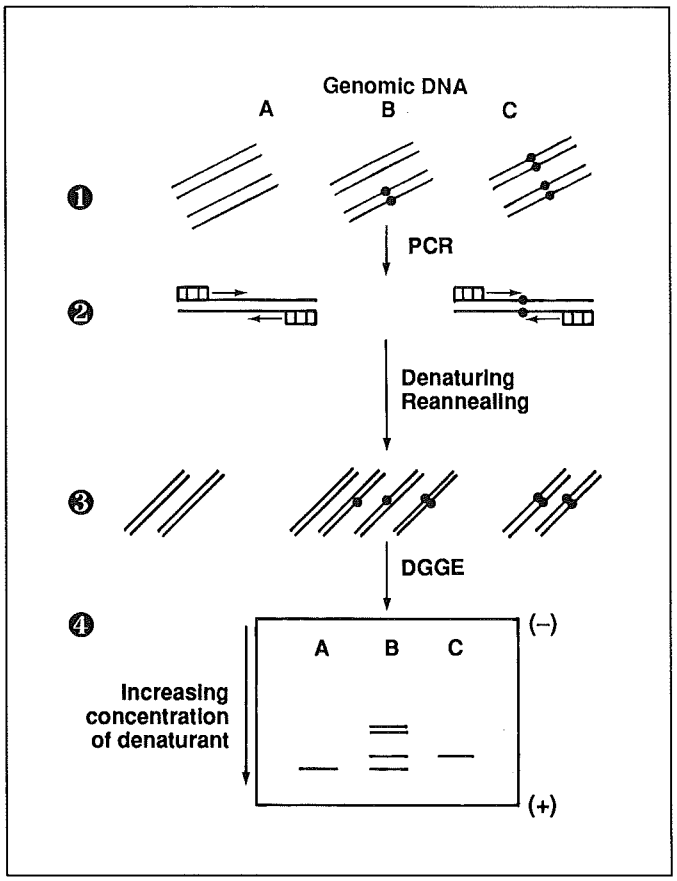
Thus, between the two groups no significant difference was observed in the rates for either mutation resulting in altered mobility and loss of enzyme activity.

Establishing permanent cell lines

Because of the importance of exhausting all possible approaches to the genetic evaluation of these children, in 1985, as the basis of future studies at the DNA or gene level, an effort was initiated to establish permanent cell lines by using Epstein-Barr virus transformation of peripheral B-lymphocytes from 1,000 family groups consisting of father, mother, and all available children (the minimum unit being trios). Five hundred of these trios were selected on the basis of one or both parents having received relatively high radiation exposures; the remaining 500 trios are controls. In this process, cell lines are allowed to proliferate approximately 500-fold ($1 \cdot 10^9$ cells) and are cryopreserved in liquid nitrogen. Intact lymphocytes and polymorphonuclear cells from all persons in the study are also preserved in liquid nitrogen in order to have a reference source of the original DNA or RNA whenever this is needed. To date, cell lines have been established from 600 trios composed of 2,000 individuals from Hiroshima and Nagasaki. The majority of the children in this project have already cooperated in the previous biochemical genetics study of mutations at the protein level.

Screening for DNA/RNA mutations

A new project, carried out in parallel with the cell line program, is the introduction and development of technologies for screening of mutations at the DNA and/or RNA levels. After precise comparative examinations of various scanning techniques and consideration of the accumulated data on several human genes, we concluded that the denaturing gradient gel electrophoresis (DGGE) of DNA:DNA duplexes produced by amplification of genomic DNA fragments with polymerase chain reaction (PCR) is the most effective technique to detect variations in DNA molecules.



Denaturing gradient gel electrophoresis (DGGE) of DNA amplified by polymerase chain reaction (PCR). 1) Three types of genomic DNA samples are shown. A: homozygous for the normal allele; B: heterozygous for the normal allele and the variant allele ( ); and C: homozygous for the variant allele. 2) Primers used in the PCR. Sequences between the two primers are amplified at least 10^6-fold by the PCR. 3) Homoduplexes of normal and variant types are produced from A and C, respectively. Two types of heteroduplexes (and), as well as a normal homoduplex and a variant homoduplex ( ), are produced from B. 4) Normal duplexes migrate faster than the variant duplexes. Heteroduplexes migrate more slowly than the two duplexes.

In "parallel" DGGE, which is suitable for a large-scale study such as ours, DNA:DNA duplexes of approximately 500 base pairs (bp) that are appropriate for detecting nucleotide substitutions will be subjected to electrophoresis on a gel in which denaturant concentration increases from the top to the bottom of the gel, the direction of electrophoresis being "parallel" with that of the denaturant gradient. When a duplex on a gel comes to a position where the concentration of denaturant is enough to denature a part of the duplex, mobility of the duplex decreases. The denaturing characteristics of the duplexes depend on their nucleotide sequences. Thus, a difference between sequences

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Biochemical Genetics

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of the duplexes results in a difference between the mobilities of the duplexes. Compared to a duplex without a mismatch, a duplex with a nucleotide mismatch starts dissociation of strands of duplexes where the concentration of denaturant is much less.

As shown in the figure on page 3, a homozygote for a normal (A) or a variant (C) allele shows the single band of a homoduplex, whereas a heterozygote (B) for a normal and for a variant allele shows four bands—one normal homoduplex, one variant homoduplex, and two types of heteroduplexes with mismatches, one of which is produced by hybridization of a normal sense DNA strand with a variant antisense DNA strand and the other produced by hybridization of the opposite combination of the DNA strands.

In our approach, a target sequence of approximately 2,500 bp in genomic DNA is amplified more than 10^6-fold by PCR, cleaved with restriction enzymes into four or five fragments of approximately 500 bp, and is examined by DGGE in a single lane on the gel. Efforts are being made to examine multiple target sequences on a single lane. Since bands on a gel are visualized by ethidium bromide, no probes or radioisotopes are necessary. Thus, the workload and the analysis time necessary for this method are minimal. We estimate that one technician can screen approximately $2.7 \cdot 10^8$ nucleotides per year with this method. For certain kinds of genes, variations in mRNA are being examined by PCR-DGGE after conversion of the mRNA to single-stranded complementary DNA with reverse transcriptase. We confirmed that the DGGE can detect not only nucleotide substitutions but also small deletions and insertions.

Considering the assumed predominance of deletional gene mutations among types of radiation-induced genetic damage, approaches to detecting deletions should be introduced and developed. The approach described above can detect deletions and insertions of less than 50 bp. This technique will also detect variations such as deletions, insertions, and rearrangements (D/I/R) of large DNA fragments since those variations will possibly be reflected as loss or change in length of the PCR products.

Detecting deletions, insertions, and rearrangements of DNA fragments

Another technique that may be used in RERF studies is that of Mohrenweiser et al., who detected the occurrence of D/I/R variants by using a modification of the usual restriction enzyme mapping strategy (*Mutat Res* 212:241–252, 1989). Variants with D/I/R were recognized by the appearance of new bands on a Southern filter. However, to detect deletions which removed the totality of single alleles, quantitative analysis of the band intensity in DNA filters is also required except for those deletions involving sex-linked genes in males. The analysis of the PCR products on two-dimensional polyacrylamide gels and two-dimensional DGGE have the potential to increase the efficiency of the search for mutations when quantitative as well as qualitative analyses are available using a computer-assisted image analyzer.

How the target sequences are selected will determine the characteristics and value of the study and the efficiency with which the study will be carried out. We suggest that the majority of the targets involve functional genes since mutations in those genes will be reflected in the quality or the quantity of the gene products and could contribute to mortality or morbidity in the children.

We estimated the numerical requirements for a study designed to demonstrate a significant difference between the mutation rates of exposed and control groups, assuming that the mutation rate is $2 \cdot 10^{-8}$ per nucleotide per generation for the functional genes and that both parents of the cell line trios have received together on average one-fifth of the doubling dose, which has been estimated recently to be between 1.7 and 2.2 Sv. The study would require that two samples of approximately $1.8 \cdot 10^{10}$ nucleotides be surveyed.

When these huge requirements and the estimated efficiency of our approach are considered, it is possible that, as in the previous studies, statistical significance may not be obtained for studies at the DNA level. Nevertheless, the data will be useful in combination with the large corpus of information already available.

We cannot emphasize too strongly how approximate these calculations are. DNA technologies are evolving so rapidly that any evaluation made today will surely be superseded within the next year or two. Next autumn a workshop will be convened to discuss the best strategy for our study, and the future of the biochemical genetics study will be planned more precisely on the basis of these discussions. ❑

News Briefs

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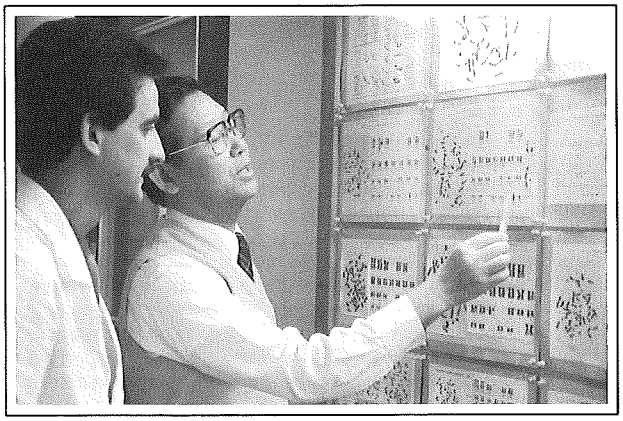

During his three-month period of study at local research facilities, José Ferreira Silva, a Brazilian pediatric surgeon, listens to RERF Genetics Department Chief Akio Awa describe RERF chromosome aberration data which can serve as a method of biological dosimetry. Silva's stay was sponsored by Hiroshima Prefecture.

0.068 Gy in sugar samples taken from two buildings 3 km from the Chernobyl nuclear power plant. The team, led by **Toshiyuki Nakajima**, reported that the tests can yield accurate results at any time after exposure regardless what method was used to refine the sugar.

An official 1988 Soviet report stated that most residents in the affected areas were exposed to 0.025 Gy or less.

✓ Highlights of the RERF Lecture Program

On 3 October, **Hiroshi Ogawa**, Aichi Cancer Center Research Institute, Nagoya, lectured on the psychology of smoking and intervention for smoking control.

Charles E. Land, National Cancer Institute, Bethesda, Md., spoke on 15 October about tobacco smoke, radiation dose, and lung cancer type: results of a binational pathology review of lung cancers among Hiroshima–Nagasaki A-bomb survivors and Colorado uranium miners.

On 24 October, **John L. McCarthy**, Lawrence Berkeley Laboratory, Berkeley, Calif., described the US Department of Energy/LBL project to develop a public use data base for epidemiologic studies of radiation effects.

Eric J. Stanbridge, University of California–Irvine, discussed tumor suppressor genes and their role in carcinogenesis on 30 October.

On 21 November, **Ruggero Montesano**, International Agency for Cancer Research, Lyon, France, lectured on the role of aflatoxin and hepatitis B virus infection in the etiology of liver cancer.

Paul Nettesheim from the National Institute of Environmental Health Sciences, Research Triangle Park, N.C., described the role of growth factors in the transformation

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Hyperparathyroidism in A-bomb Survivors: An Update

Results relating hyperparathyroidism to A-bomb exposure have stimulated close monitoring for this condition during RERF's Adult Health Study examinations. New data are presented.

by Saeko Fujiwara
Department of Clinical Studies

Since the mid-1970s, hyperparathyroidism (HPT) induction has been associated with radiotherapy of the head and neck region. Interestingly, during the past few years, results from RERF's Adult Health Study (AHS) biennial examinations have begun to reveal a possible relationship between HPT and A-bomb radiation exposure, thus suggesting that relatively low-dose exposure may also induce this disorder.

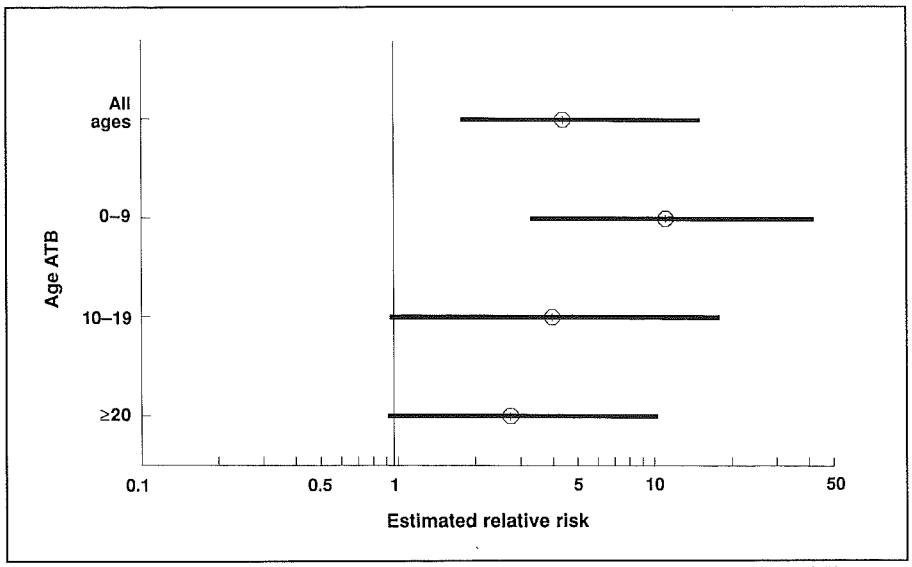
A study conducted at RERF from August 1986 through July 1988 on 4,675 persons aged 41 or older showed that HPT was more prevalent among A-bomb survivors who received higher radiation doses (test of linear trend, $p < .001$), and the effect of radiation exposure was greater among individuals exposed at a younger age ($p < .05$) (Fujiwara et al., RERF TR 8-90). The relative risk of developing HPT at 1 Gy was 4.38 in all age groups combined, 11.1 in those 0–9 years of age at the time of the bombing (ATB), and 2.75 in those ≥20 years old ATB (see figure). Although no difference in radiation effect between the sexes was noted, females exhibited a threefold higher overall prevalence of HPT than males.

Here we will summarize new HPT data accumulated since August 1988.

Diagnosis of HPT

HPT, a generalized disorder of calcium and phosphate metabolism resulting from an increased secretion of parathyroid hormone, is caused by adenoma in approximately 82–85% of cases, by hyperplasia in 15%, and by cancer of the parathyroid gland in a small percentage of cases.

In 1986, acquisition of an autoanalyzer enabled cost-effective blood chemical measurements to be routinely undertaken as part of RERF's biennial AHS examinations. Thereafter, HPT diagnosis has been determined biochemically, based upon the presence of consistent hypercalcemia (Ca ≥ 10.3 mg/100 ml), accompanied by an elevated level of serum parathyroid hormone (≥520 pg/ml).



Among AHS participants, the estimated relative risk of hyperparathyroidism at 1 Gy is shown by age at the time of the bombing (ATB), 1986–1988 (point estimates and 95% confidence interval for the relative risk).

New data since August 1988

Persons newly suspected of having HPT were found more frequently in the ≥1 Gy group. From August 1988 through July 1990, 12 subjects were thought to have HPT, including one female who had been in utero ATB. At a previous HPT screening conducted from 1986–88, six of these individuals had exhibited borderline levels ranging between 9.9 and 10.2 mg/100 ml of serum calcium.

The number of HPT cases by thyroid dose category is shown in the table.

We are somewhat concerned about the characteristically fluctuating nature of serum calcium levels that in borderline HPT cases may be below the "cutoff" point of 10.3 mg/100 ml at the time of screening. These AHS participants are not requested to return within a few days for a more conclusive parathyroid hormone screening and are therefore lost to HPT follow-up until the next cycle of biennial AHS examinations. If the prevalence rate of such borderline cases is higher among the high-dose groups than in the non-exposed group, we may be underestimating the effects of radiation exposure.

To further investigate the significant linear relationship between radiation exposure and the serum calcium level found in the AHS participants covered in the 1986–1988 survey (common slope, 0.073 ± 0.018 mg/100 ml/Gy), we decided to measure the PTH level of 1,600 subjects selected at random from Hiroshima AHS participants (Fujiwara et al., RERF RP 2-89). A more definitive HPT indicator, PTH screening is costly and is usually undertaken at RERF only when clear-cut hypercalcemia is diagnosed.

From May 1989 to August 1990, we found four patients who showed PTH levels indicative of HPT, i.e., >1,000 pg/ml. They had not been suspected of having HPT at either the 1986–1988 or 1988–1990 screening exams because they showed borderline calcium levels ranging from 10.0–10.1 mg/100 ml, thus indicating that some HPT cases might have an upper but normal range of serum calcium. As noted with the recent hypercalcemia cases, the thyroid dose for three of these individuals was greater than 1 Gy.

Thus, using two biochemical techniques, HPT is being diagnosed even in asymptomatic A-bomb survivors. Follow-up for HPT diagnosis in borderline hypercalcemia patients will help determine the clinical features of such cases and whether they were actually induced by radiation exposure. ❑

AHS hyperparathyroidism cases: 1986–1990

Thyroid dose (Gy)	1986–88	1988–90
0–0.009	3 (1583)*	0
0.01–0.499	4 (1353)	3
0.5–0.999	5 (514)	0
≥1.00	7 (498)	7
Dose unavailable	3	1
In utero	0	1
Total cases	22	12

*No. of AHS participants tested for serum calcium.

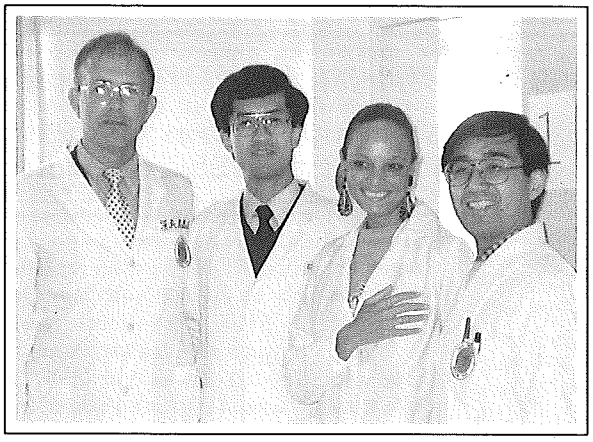
RERF Physicians Journey to the Soviet Union

At a time of both economic and political crisis in the Soviet Union, acquiring the necessary resources to provide medical care for those affected by the Chernobyl accident has begun to take on a greater sense of urgency. The firsthand experience of three RERF physicians dispatched to join the first group of the International Atomic Energy Agency's (IAEA) Health Effects Research Team confirms the ongoing need for international generosity and resource commitment.

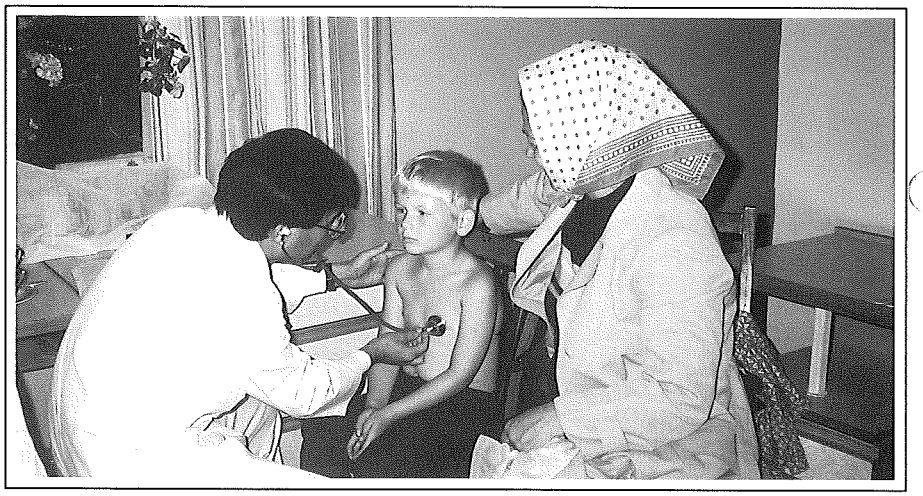
The Health Effects Research Team, only one of five research teams established by an IAEA-convened advisory committee headed by RERF Chairman **Itsuzo Shigematsu** (see *RERF Update* 2(2):1–2, 1990), conducted medical examinations in affected areas during September and October 1990.

From RERF, **Hideo Sasaki**, assistant chief of the Department of Clinical Studies, and the chiefs of the Division of Clinical Laboratories, **Shizuyo Kusumi** (Hiroshima) and **Naokata Yokoyama** (Nagasaki), participated in the health examination of residents of the Ukraine Republic from 31 August to 16 September. Headed by Professor **Fred Mettler**, Department of Radiology, the University of New Mexico, this first group consisted of 10 members from various countries, including Japan, the United States, and the Soviet Union, as well as IAEA personnel from Morocco and South Africa.

S. KUSUMI



At left, part of the IAEA Health Effects Research Team: (from left) Fred Mettler, Naokata Yokoyama, Jane Hendricks (IAEA staff), and Hideo Sasaki. Shown below, one of the more than 30 patients per day seen by Sasaki during his two-week stay in the Ukraine. All team members experienced similar workloads as residents willingly participated in the IAEA-sponsored examination program.



"We felt that anxiety about the effects of radiation exposure was very deeply rooted in the residents of all districts we visited, irrespective of the presence or absence of contamination," commented Sasaki. "Consequently, the residents were expecting much from the research team, and we were always wholeheartedly welcomed wherever we went.

"During our examinations, various questions arose, and we tried the best we could to give as detailed an explanation as possible," he recalled. "But the language barrier prevented us from communicating freely."

Surprised by the conditions that confronted him and his colleagues, Sasaki said, "Financial exhaustion in the Soviet Union was greater than we had expected and insufficient nutrition due to a shortage of food also seemed to have exerted grave effects on the health of the residents."

A detailed report containing the IAEA Health Effects Research Team's findings is scheduled for publication in mid-1991. ❑

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of airway epithelial cells on 30 November.

On 11 December, **Kelly H. Clifton**, University of Wisconsin–Madison, lectured on the biology of mammary and thyroid clonogens, the cells of origin of experimental radiogenic cancer.

✓ *Enola Gay* Log Sold

On 7 December, Christie's, a New York auction house, sold the flight log kept by the *Enola Gay*'s navigator for 6 August 1945, the day the B-29's crew detonated the "Little Boy" atomic bomb over Hiroshima. The log carries the entry "bomb away" for 9:15 a.m., which was 8:15 a.m. local time.

✓ Hiroshima Area Institutions Outline Outreach Activities

In late November, 13 representatives of medical research institutes in Hiroshima met to plan activities for 1991, including establishing criteria for accepting doctors from overseas for training as well as for dispatching Japanese medical experts to areas of interest, preparing a comprehensive resource document on A-bomb survivor medical treatment, conducting surveys on the dissemination of information, and sponsoring lectures. Financial aid for two- or three-day inspection tours of local facilities by foreign scientists was also discussed.

✓ RERF Asked to Assist in Japanese Government's Efforts

In September, Japanese Foreign Minister **Taro Nakayama** and former Soviet Foreign Minister **Eduard Shevardnaze** signed an agreement calling for Japanese cooperation in the medical care of those affected by the Chernobyl power plant accident, as well as in research areas related to the event. The Japanese government will assist in efforts to assess the damage related to the accident, the health of the exposed people, and the management of their medical care.

To begin developing a cooperative program, experts from both countries, including RERF Chairman **Itsuzo Shigematsu** and RERF Permanent Director **Yutaka Hasegawa**, met in early December in Tokyo. The next meeting of this group will be held in the Soviet Union early in 1991.

Through the World Health Organization, the Japanese government is to offer the Soviet government ¥2.6 billion (about US $20 million) in financial aid for the medical relief of those affected by the Chernobyl accident—¥2 billion (US $15.4 million) for medical equipment and ¥600 million (US $4.6 million) for medical technology assistance.

In addition, the Japanese Science and Technology Agency has announced plans to perform a large-scale survey of radiation effects due to the accident. It is likely that the survey will be conducted jointly by Japan and the USSR, and that specialists from RERF will participate in the effort. ❑

Recent Scientific Publications

Approved Technical Reports

Gamma-ray- and fission-neutron-induced micronuclei in PHA-stimulated and unstimulated human lymphocytes. S Ban, MP Donovan, JB Cologne, S Sawada. **RERF TR 9-90.**

Two groups of normal human blood cells, one stimulated with phytohemaglutinin (PHA) for 24 hr (G_1-S phase of the cell cycle) and one unstimulated (G_0 phase), were irradiated with Co-60 gamma rays or Cf-252 radiation. A comparison of radiation-induced micronucleus frequencies showed that the high-dose-rate gamma rays were more effective in inducing micronuclei than were low-dose-rate gamma rays. In the cells exposed to low-dose-rate irradiation, there was little difference between the frequency of micronuclei in the G_0 phase and the G_1-S phase. However, cells in the G_1-S phase were more sensitive than G_0-phase cells to high-dose-rate gamma rays. The relative biological effectiveness of Cf-252 neutron irradiation measured in micronucleus assays was consistent with the value obtained for the lethal effect of Cf-252 on cultured cells.

TCR mutant T cells: a novel marker for biological dosimetry of recent radiation exposure. S Kyoizumi, M Akiyama, S Umeki, Y Kusunoki, Y Hirai, N Nakamura, K Endoh, J Konishi, M Sasaki, T Mori, JB Cologne. **RERF TR 10-90.**

This study reports a method of biological dosimetry based on measurements of the frequency of mutant T lymphocytes defective in T-cell receptor (TCR) gene (α or β) expression using a two-color flow cytometric technique. A significantly increased mutant frequency was observed for patients who had received Thorotrast, a contrast medium containing Th-228, formerly used for radiodiagnosis. In addition, thyroid disease patients treated with I-131 showed a highly significant dose-related increase in mutant frequency. In contrast, proximally exposed atomic bomb survivors (estimated doses of ≥1.5 Gy) did not show a significantly increased mutant frequency compared to distally exposed individuals (estimated doses of <0.005 Gy). Two important characteristics of the present TCR mutant T-cell assay are that it could be used as a biological dosimeter for measuring recent radiation exposures separately from accumulated previous exposures, and that it is practical for use in large-scale screenings of populations exposed to radiation.

Approved Research Protocols

Cryopreservation of blood cells from Hiroshima and Nagasaki AHS participants. M Akiyama, N Nakamura, Y Hirai, Y Kusunoki, T Seyama, K Kodama, S Kusumi, K Hamatani, M Abe, T Honda, T Matsuo, N Yokoyama, K Shimaoka. **RERF RP 2-90.**

This proposal advocates initiating a program of cryopreservation of blood cells from all Adult Health Study participants (about 7,300) in Hiroshima and Nagasaki. This will ensure that appropriate materials will be available for future studies of the late effects of human exposure to atomic bomb radiation, and will allow the exploitation of potential future technological advances and scientific discoveries.

The association of serum cholesterol with noncardiovascular mortality and morbidity in the Adult Health Study population. H Sasaki, S Akiba, K Kodama, FL Wong, S Fujiwara, N Yokoyama, F Kasagi, M Soda. **RERF RP 3-90.**

A J-shaped dose response between serum cholesterol level and total mortality has been frequently reported in several cohort studies. The increase of total mortality with serum cholesterol level in the dose-response curve is mainly attributable to coronary heart diseases, in which serum cholesterol plays a major role in atherogenesis. An inverse relationship between cholesterol and mortality from all causes at relatively low cholesterol levels is suspected to involve a variety of diseases.

The study proposed here will examine the relationship of serum cholesterol to the mortality and morbidity of noncardiovascular diseases in general, and for major diseases including cancer in the Adult Health Study population (AHS) using the accumulated information from the AHS examinations, the tumor registries, and death certificates. If an association is found, we will attempt to determine (a) when the cholesterol changes first appeared in prediagnostic sera, and (b) what factors, if any, modify the association between serum cholesterol and mortality and morbidity.

Establishment of a method for HLA-DQ and DP gene typing using the polymerase chain reaction. T Hayashi, Y Hirai, Y Kusunoki, N Nakamura, M Akiyama. **RERF RP 4-90.**

A pilot study is proposed to establish a method of human leukocyte antigen (HLA) gene typing that employs the polymerase chain reaction (PCR) using DNA extracted from lymphocytes instead of conventional HLA typing using antisera.

Conventional HLA typing requires the combined use of various antisera, and it has not been suitable for large-scale applications with A-bomb survivors because of the extensive time, labor, and costs involved. However, a recently developed typing method that uses PCR now makes it possible to conduct HLA typing very rapidly, at low cost, on a large number of samples. The aim of this pilot study is to establish this gene typing method at RERF. In addition, the experimental conditions for the microassay using peripheral blood samples from A-bomb survivors will be examined.

Primary liver cancer incidence study among atomic bomb survivors, Hiroshima and Nagasaki, 1958–87. T Fukuhara, H Itakura, M Yamamoto, S Tokuoka, K Mabuchi, S Akiba, T Seyama, N Nakamura, M Akiyama, JB Cologne, M Soda, M Tokunaga, GW Beebe. **RERF RP 5-90.**

The proposed study is an investigation of liver cancer incidence among the RERF Extended Life Span Study population during the period 1958–87 under the RERF guidelines for site-specific cancer incidence studies. Liver cancer has been chosen for investigation at this time because: (a) it is a major site of cancer with a definite etiologic link to alpha irradiation but no demonstrable link as yet to low-LET irradiation; (b) its incidence apparently differs significantly between Hiroshima and Nagasaki; and (c) the latest review, by Asano et al., for the period 1961–75 included 128 confirmed primary liver cancer (PLC) cases.

By 1987 there were over 1,100 potential cases for review, of which more than 950 were coded to liver cancer (ICD-O topography = 1550). A diagnostic review by pathologists will identify PLC on the basis of pathology materials and clinical information including echo, CT, scintigram, and tumor markers from autopsy and clinical records identifiable from tumor and tissue registries. The pathology review will extend to the histologic classification of identified PLC, to the diagnosis of any accompanying liver cirrhosis, and to testing for hepatitis-B virus (HBV) markers. For each autopsied PLC case, two matched controls will be selected from the ABCC–RERF autopsy series. Case-control analysis will be used for an evaluation of the influence of HBV on the relationship between radiation dose and PLC, the major interest of the study.

It is hoped that the study, which builds on earlier investigations at ABCC–RERF, will also lay the groundwork for the continuing investigation of this major tumor site.

Approved Commentary and Review

A nested case-control approach to interactions between radiation dose and other factors as causes of cancer. CE Land. **RERF CR 1-90.**

Often a nested case-control study is the most practicable approach to estimating the interaction of two cancer risk factors in a large cohort. If one of the factors has already been evaluated for the entire cohort, however, more information is already available about its relationship to risk than could be obtained from a nested study. A modified case-control approach is proposed, in which information about the second, unknown factor is sought for cases and controls matched on the first factor. The approach requires, for interaction models other than the multiplicative, a nonstandard analytical approach incorporating cohort-based information about the first factor. The problem is discussed in the context of breast cancer risk in a defined cohort of female Japanese atomic bomb survivors, in relation to radiation dose and reproductive history.

continued on next page

Recent Scientific Publications

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Publications in the Open Literature

Radiosensitivity of CD4 or CD8 positive human T-lymphocytes by an in vitro colony formation assay. N Nakamura, Y Kusunoki, M Akiyama. *Radiat Res* 123:224–7, 1990. (RERF TR 16-89)

Approaches to DNA methods for the detection of heritable mutations in humans. C Satoh, K Hiyama, N Takahashi, M Kodaira, JV Neel. In: *Mutation and the environment. Part C.* Edited by ML Mendelsohn, RJ Albertini. New York, Wiley-Liss, 1990. pp. 197–206

Trend of coronary heart disease and its relationship to risk factors in a Japanese population: a 26-year follow-up, Hiroshima/Nagasaki study. K Kodama, H Sasaki, Y Shimizu. *Jpn Circ J* 54:414–21, 1990.

Commentary. Childhood leukemia at Sellafield. S Abrahamson. *Radiat Res* 123:237–8, 1990.

Cigarette smoking and cancer mortality risk in Japanese men and women—results from reanalysis of the six-prefecture cohort study data. S Akiba, T Hirayama. *Environ Health Perspect* 87:19–26, 1990.

Calculating excess lifetime risk in relative risk models. M Vaeth, DA Pierce. *Environ Health Perspect* 87:83–94, 1990. (RERF CR 3-89)

Allowing for random errors in radiation dose estimates for the atomic bomb survivor data. DA Pierce, DO Stram, M Vaeth. *Radiat Res* 123:275–84, 1990. (RERF TR 2-89) ❑

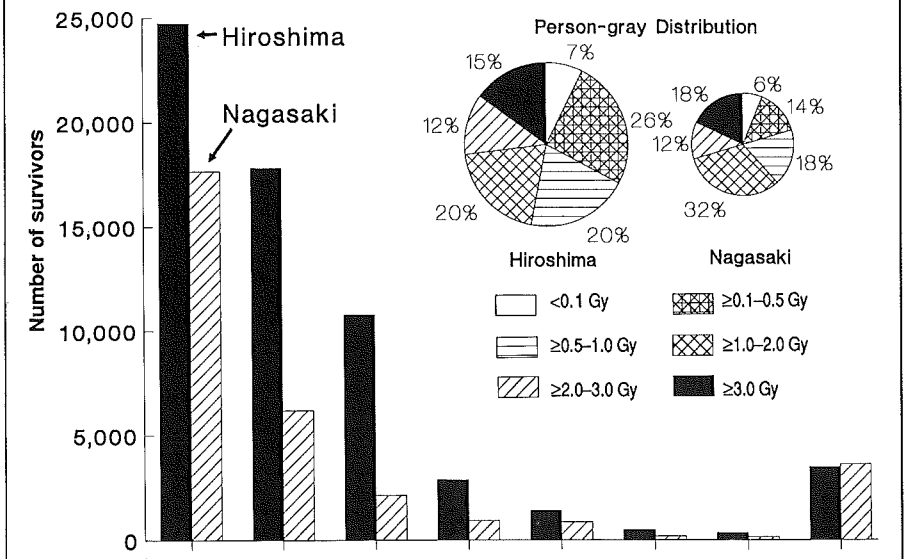
Facts & Figures

The LSS Cohort: A-bomb Kerma Distribution

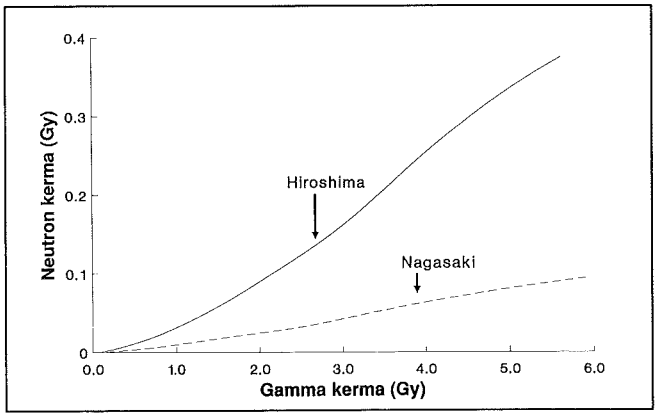
We present kerma distribution, person-gray distribution, and average relative composition of gamma and neutron dose for Life Span Study members who were in city at the time of the bombings (Hiroshima: 61,936, Nagasaki: 31,733) with complete mortality follow-up data by city. Although doses to specific organs are used in estimating risk, the figures below are based on free-in-air kerma adjusted for external shielding.

The histogram also shows the number of survivors for whom DS86 estimates cannot be calculated. The inset displays the person-gray distribution, with the <0.01 Gy and 0.01–0.1 Gy categories combined and the "unknown" category excluded (total person-gray: Hiroshima—9,802.3; Nagasaki—3,697.6).

Note that survivors with kerma in excess of 0.5 Gy (8% of total survivors with dose estimates) account for most of the person-gray in the LSS (71%), and thus greatly impact risk estimates. ❑



Kerma (Gy)	<0.01	≥0.01–0.1	≥0.1–0.5	≥0.5–1.0	≥1.0–2.0	≥2.0–3.0	≥3.0	Unknown
Hiroshima	24,737	17,836	10,787	2,870	1,409	482	338	3,477
Nagasaki	17,671	6,193	2,138	921	865	182	143	3,620



At left, information on average neutron kerma as a function of average gamma kerma in Hiroshima and Nagasaki. Neutrons account for a small fraction of the total kerma in both cities, but the contribution of neutrons to total kerma is much less in Nagasaki than in Hiroshima.

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.

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