

EXTENDED ABSTRACTS

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The 1998 American Statistical Association Conference on Radiation and Health was held in San Diego, CA, June 14–17, 1998. This year marks the 13th convening of this conference. The conference has grown to include scientists from many countries and disciplines involved in research on the health effects of radiation (including statistics, epidemiology, radiation biology, health physics and genetics).

The 1998 conference was an opportunity to review our current state of knowledge regarding the health risks from radiation exposure with a focus on low-dose and low-dose-rate effects. The program addressed the following areas of importance in public health:

1. The contribution of epidemiology to our understanding of the effects of exposure to low-dose ionizing radiation on human health.
2. The links between radiobiology and radiation epidemiology.
3. Radiosensitive populations: significance for risk estimation and radiation protection.
4. Indoor radon and the risk of lung cancer.
5. Dosimetry at low doses.
6. New paradigms for low-dose radiation response.

The extended abstracts that follow provide an excellent summary of the proceedings of this 13th ASA Conference on Radiation and Health.

I. THE LINKS BETWEEN RADIOBIOLOGY AND RADIATION EPIDEMIOLOGY: CAN RADIATION EPIDEMIOLOGISTS AND RADIOBIOLOGISTS HELP EACH OTHER?

Chair: Evan B. Duple, *National Academy of Sciences*

Dose Response, Temporal Response and Biologically Based Models in the Bridge between Human and Rodent Carcinogenesis

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Several concepts in carcinogenesis provide the central framework for my views. In roughly temporal order, and with apologies for historical and academic incompleteness, these are:

1. The idea of Boveri (1) of mitotic instability, and the associated ambiguity that instability can be the cause or the result of the cancer process.
2. The idea of Berenblum and Shubik (2) of a combined mutation-like and proliferation-like or promotional mechanism in rodent skin carcinogenesis.
3. The concept that cancer is clonal (3) and is an interrupted development of stem or stem-like cells rather than a de-differentiation from something more mature.
4. Armitage and Doll's elaboration (4) of the remarkably good fits of human cancer rates to age to the roughly 6th power.
5. The compelling evidence from the National Toxicology Program (5) that roughly half of the rodent carcinogens are not mutagens, contrasted to my softer impression that almost all of the noncontroversial human carcinogens are mutagens.
6. The elegantly simple, but not generally applicable, homozygous loss of normal RB in the causation of human retinoblastoma (6).
7. The wealth of oncogenetic change that is now known to characterize human malignancy, including suppressors, oncogenes, amplifications and repair defects.
8. The relatively small effect on cancer rate from single-copy knockout of suppressor genes in rodents (7).
9. The exquisitely linear no-threshold response of the incidence of radiation-induced cancer in the atomic bomb survivors, and the corresponding evidence that background and induced cancers are biologically indistinguishable and roughly proportional by type (8).

These concepts, particularly those inclusive of items 1–6, led to the widely used conceptual and mathematical model that cancer involved two specific mutations with intervening growth (9). This model is remarkably flexible, so much so that it is essentially nonconstraining on the results of most cancer studies. It can even fit the age to the power 6 relationship for human cancer, although it offers no explanation for why the relationship is so prevalent. The model may not be consistent with the studies in knockout mice (item 8 above), and it raises serious questions about the lengthy delays of adult cancer in long-lived species.

At the opposite (mutational) extreme is a recently introduced model (10) to explain the current results in atomic bomb survivors. This purely mutational model argues that background incidence of solid cancer is due to the gradual accumulation of k hits in the oncogene and suppressor gene targets of stem cells. The first viable stem cell to collect the k mutations is the founding cell for the first cancer in that person, while later accumulations in other stem cells provide "backup" cancers should the first process fail, or should the first cancer be cured. Given that background mutations, whether caused internally or externally, are likely to be a time-based process, the overall effect is that cancer rate will follow age to $k - 1$ power. In the corresponding radiation-related cancers, the acute radiation exposure introduces somatic mutations as a function of dose, and these cumulate in the stem cells, displacing whichever is the next mutation to be cumulated by time. This results in a radiation-en-

hanced cancer process having a mutation-based dose response with a rate that follows age to the $k - 2$ power. The types and the biological details of the radiation-related cancers are likely to differ very little from background. The model fits well to the background and radiation-related solid cancers in the atomic bomb survivors and suggests both that the cancer risk will persist through the lifetime of the survivors and that there is no evidence of increased sensitivity in those exposed as children.

Is it conceivable that human cancer is purely mutational, i.e. has no promotional component? Probably not, and the simplest evidence for this is that hormonal cancers, particularly breast and thyroid cancer, do not show the age to the $k - 1$ power relationship. Other evidence is the well-known national differences in organ preference which seem not to have a genetic basis but rather to be due to dietary, cultural or other geographic factors. Curiously, it is cancer type that changes and not cancer rate when Japanese migrate to the U.S., suggesting that the promotional component is influencing the "mutational race" between stem cells of one organ and another (e.g. stomach and colon) without changing the overall risk. It would be interesting to have the Armitage-Doll plots of this process.

A model for carcinogenesis in humans that is dominated by mutation and involves accumulation of successive mutations over a lifetime essentially converts the low-dose controversy from the shape of the cancer dose responses to the shape of the dose responses for somatic mutations. For the latter, the pattern at low doses is inherently linear and no-threshold, and is potentially modifiable by the conditions of repair and cell kinetics. Processes like adaptation are capable of reasonable dissection as mutational mechanisms (and hence are accessible to study *in vitro* and over a relatively short time), unlike carcinogenesis, which in the human is remarkably inaccessible. In addition, the very notion of cumulating stem cells carrying increasingly understood mutations is a tantalizingly available target for cancer prevention.

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Extrapolation of Risk Estimates across Species

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Current Use of Experimental Animal Data for Estimates of Risk

There has been a firm reluctance to accept the use of direct risk estimates of cancer induction by radiation obtained from animal experiments in the estimation of risks in humans. However, data from animal experiments are used in the derivation of equivalent doses in tissues, H_T , for radiological protection purposes and given by the expression

$$H_T = \sum_R w_R \cdot D_{T,R},$$

where w_R is the radiation weighting factor, selected for the type and energy of the radiation and $D_{T,R}$ is the absorbed dose averaged over a tissue, T , due to radiation R .

The w_R 's are selected from the relative biological effectiveness (RBE) factors from experimental studies such as tumor induction in mice. Quality factors are similarly based on RBEs.

The risk estimates for radiation-induced cancer at low doses or low dose rates are based on the equivalent doses and the dose-response relationships. To estimate the risk coefficient at low doses, an adjustment is made of the risk estimates obtained at high doses and high dose rates by applying a dose and dose-rate effectiveness factor (DDREF). This factor is selected based on animal data. Thus two ratios, RBEs and DDREF, derived largely from animal experiments, are accepted for the estimates of risk in humans. The use of w_R 's and DDREF are necessary because there are no data for risk of induction of cancer by protons, neutrons and other high-LET radiations. But why are ratios of dose-effect responses acceptable and yet it is not considered possible to extrapolate the component dose-effect responses to risk estimation in humans?

If these ratios are considered suitable for extrapolating across species, it should be done in the most appropriate manner. Take, for example, DDREF, which contributes more to the uncertainty of lifetime risk of excess cancer mortality than any other factor that has been examined (1). The choice of a single value for DDREF may have the advantage of simplicity, but if unweighted, hardly reflects the considerable differences between tissues. Since at low doses excess mortality in mice exposed to radiation is accounted for by excess tumors, the effect of dose and dose rate could be assessed using life shortening. It would retain the advantage of a single figure and would approach the question of weighting for tissue-dependent differences in susceptibility. Obviously, the appropriateness of the weighting would depend on how similar the spectra of tumors in the mouse and human populations were. Another disadvantage of the present system is the large errors in the components of the ratio. There is no more clear-cut end point than death; even I can diagnose it with 100% accuracy. Furthermore, the initial response curves for both high and low dose rates are linear. A similar argument can be made for the derivation of w_R 's, at least in the case of neutrons, provided the effect of the disparity of body size on the quality of the radiation in deep tissues is taken into account.

Methods of Extrapolation

Despite the use of animal data to derive DDREF and RBEs, no attempt has been made to use direct estimates of risk of radiogenic cancer. There is the perception that tumor rates are higher in mice than in humans. It is clear that the life spans and the latent periods of induced tumors are very different in the two species. The great disparity in size and therefore presumably in the number of cells that are targets for cancer induction is considered a stumbling block to extrapolation. However, the more information that is revealed about the genomes of mouse and man, the more evidence there is that the genes involved in cell proliferation and its control and in differentiation have been conserved. This is the reason that transgenic and "knockout" mice have become the experimental models par excellence for cancer research in general. The simi-

larities in carcinogenesis in the two species gives some encouragement that the search for methods of extrapolation is not completely cockeyed.

A small number of groups have taken different approaches to the problem. In the case of internal emitters, there was a pressing need to estimate the risk of cancer in workers exposed to plutonium for which there were no data for humans. Since there were data for cancer risk from radium, a ratio similar in concept to RBEs was used and termed Toxicity Ratio (2). Making some assumptions, the risk of bone cancer due to ^{239}Pu in humans could be calculated from the risk observed in radium dial painters from the relationship

$$\left[\frac{\text{Risk from } ^{239}\text{Pu}}{\text{Risk from } ^{226}\text{Pu}} \right] \text{ in man} \approx \left[\frac{\text{Risk from } ^{239}\text{Pu}}{\text{Risk from } ^{226}\text{Pu}} \right] \text{ in animals.}$$

A different approach used a Bayesian method for integrating the findings from studies in experimental animals and in humans to obtain a risk estimate for radionuclides for which risk estimates in humans are lacking (3). While the confidence limits are quite broad in the case of ^{239}Pu , the approach has found some favor.

From a study involving six mouse strains and nine tumor sites, it was concluded that susceptibility for the induction of solid cancers by radiation was related to the natural incidence of the specific cancer type (4). Furthermore, the estimates, based on the relative risk model, were superior, at least, in the majority of solid tumors but not leukemias. A preliminary examination suggested relative risk estimates for tumors in mice were comparable to those in humans. There are obvious concerns in such approaches, including the fact that in the mouse experiments the exposures were all at one age, whereas the humans were exposed over a range of ages. Another important difference is the marked radiosensitivity of the ovary in the mouse and the consequences of ovarian damage.

For many years Sacher, Grahn and their colleagues (5) and more recently Carnes and his colleagues (6) have studied the factors influencing longevity and radiation-induced life shortening. In the earlier studies, mortality ratio or relative risk was determined for a number of mouse strains and found to be the most suitable measure of determining excess tumor risk from low doses of radiation. It was suggested that the use of a scaling factor of 30:1 mouse:human to account for differences in life span made it possible to translate coefficients from the mouse studies to the human experience.

In recent analyses, it has been found that scaling to adjust for species-dependent differences in life span made predictions of life shortening in irradiated beagles possible using data for risk estimates in mice (6). Currently, this approach is being applied to the prediction of life shortening in humans based on the data for mice. Validations will depend on the congruence of the values predicted and those obtained from the study of the atomic bomb survivors.

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Did Radiobiology Play a Useful Role in the Recent BEIR VI Report?

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It has long been clear that extrapolation of measured radiation risks to environmentally relevant situations requires information that epidemiological studies, on their own, cannot provide. In principle, this "extra", non-epidemiologically based, information required for risk extrapolation is the domain of radiobiology, either experimental or theoretical. In practice, however, this has rarely been the case. For example, although the first five National Academy of Sciences BEIR reports on radiation risk estimation have included chapters on radiobiology, the contents of these chapters have played a limited role, if any, in the risk estimation parts of these reports.

At least in principle, there is a wide range of tools that are available for analyzing and interpreting quantitative radiation epidemiological data. At one extreme, most previous analyses of quantitative data on radiation-induced carcinogenesis, such as those in the first five BEIR reports, have been based largely on empirical, descriptive modeling of epidemiological data. At the other extreme, there has been some recent interest in analyzing epidemiological data using so-called biologically motivated models, designed to provide realistic quantification of all the relevant steps from energy deposition to the appearance of, say, cancer. In this context, the parameters of the model have a biological interpretation and can, in theory, be evaluated directly from experimental data.

Neither of these extreme situations (no radiobiological input used, or complete radiobiological mechanisms assumed) is currently satisfactory. A middle way is discussed here which, it is suggested, was useful in the recent BEIR VI report on radon risk estimation (1); in this approach, radiobiological data or concepts can be used to guide empirical epidemiological analyses in specific areas where there is uncertainty, but no general model for, say, radiation-induced carcinogenesis is assumed.

This hybrid approach can work in either direction, with radiobiology facilitating radiation epidemiology, or *vice versa*. In one direction, radiobiology can identify areas of uncertainty in the model that need elucidation from data for humans, and specific epidemiological studies can be designed to address these uncertainties. Conversely, data from epidemiological studies may also point to specific areas of uncertainty that can be addressed through studies of mechanisms.

This approach is illustrated with reference to the recent BEIR VI Report on the health effects of residential exposure to radon (1). In that the BEIR VI risk estimates were anchored to epidemiological data from miners exposed to radon, several key areas of uncertainty were recognized that could not be resolved by reference to epidemiological data alone. Among these were

1. Extrapolation from high to very low radon exposures.
2. Extrapolation from higher to lower radon exposure rates.
3. Interaction of damage from radon progeny with that from other agents.
4. Individual variations in susceptibility to radiation-induced cancer.

All of these issues, which are central to generating a final risk model, were assessed primarily based on current understanding of the underlying radiobiology, rather than by "blindly" assessing the epidemiological data alone. The resulting risk model for residential radon exposure is probably more credible than one which could be obtained by a purely empirical description of the data for the miners. Two examples follow of this hybrid approach to risk estimation, as applied in the BEIR VI report to the problem of residential exposure to radon:

The Inverse Dose-Rate Effect for Radon

When a given dose of low-LET radiation is protracted, whether by lowering the dose rate or through increased fractionation, the biological effect either is unchanged or decreases, because of the possibility of repair of sublethal damage during the irradiation. However, it has become increasingly clear that densely ionizing (high-LET) radiations such as neutrons and α particles exhibit an "inverse dose-rate effect" for oncogenic end points. Specifically, for a given dose or exposure, as the dose rate is lowered, the probability of oncogenesis increases. This inverse dose-rate effect clearly involves protraction effects other than those of repair of sublethal damage—this latter being the dominant effect of protraction at low LET.

The role of dose rate as a modifier of the dose-response relationship for miners exposed to radon has long been considered in epidemiological studies. The first evaluations of the data for the Colorado Plateau uranium miners, in the early 1970s, found no significant variation of the exposure-response relationship with exposure rate. However, a later (1977) follow-up demonstrated a significant inverse dose-rate effect, with the lung cancer risk at equal WLM exposure greater in miners exposed at lower exposure rates. These results supported an analysis of Czech miners, which also reported greater effects among miners, exposed at lower exposure rate.

Independent of these early studies of miners exposed to radon, this inverse dose-rate effect was observed from the late 1970s in experiments in laboratory animals for carcinogenesis induced by neutrons and charged particles, and for life-shortening experiments with neutrons. These results in animals prompted corresponding studies with quantitative *in vitro* oncogenic transformation systems exposed to neutrons and charged particles.

In turn, these laboratory results stimulated theoretical studies of possible mechanisms. Although the exact mechanisms were not, and still are not, fully elucidated, some general—and model-independent—principles became clear which must control the general patterns of how the effect changes with differing doses and dose rates. Specifically, at low doses of high-LET radiation, where target cells (or small groups of target cells) are subject to an average of much less than one α particle (or neutron), there can be no dose-rate effect of any kind, as the target cells would not be "aware" of any dose protraction. At higher doses, of course, dose-rate effects are possible.

In the late 1980s, again independent of the radiobiological investigations, stronger evidence for inverse dose-rate effects in studies of miners became apparent. While the inverse dose-rate effect was clearly evident in these epidemiological studies of miners, what did not emerge from these analyses was its dose dependence. Generally speaking, at lower exposures, the statistics become poor, and so, unless one was looking for an exposure dependence for the inverse dose-rate effect, one would be unlikely to see it. In the light, however, of the radiobiological analyses (e.g. 2), the authors of what was then the largest study of miner cohorts re-examined their data (3) and did observe a decrease in the inverse dose-rate effect with decreasing dose, until it essentially disappeared at exposures corresponding to less than one α particle traversing target lung cells.

Such a finding is relevant to the extrapolation of risk from data for miners, where there is generally an inverse dose-rate effect, to residential exposures, where one would not be expected. In light of this understanding, the BEIR VI model (a) explicitly allows dose-rate effects to depend on exposure and (b) puts greater emphasis on assessing the risk projection model using only data for low exposures where the effect is minimal, and assessing the risk projection model using only data for residential exposures.

Low-Dose Extrapolation

A common problem in epidemiology is to define the dose-effect relationship at low doses, and those who assess risk face the even more daunting task of extrapolating to low doses, where there are no useful data. Realistically, with current techniques, radiation epidemiology stud-

ics on their own are unlikely to provide quantitative conclusions at residential radon exposure levels below, say, 150 Bq/m³.

A common assumption is that, at low doses, a linear relationship is appropriate down to arbitrary low doses—a concept often described as “linear no-threshold”. Is this linear extrapolation correct? The biophysical rationale involves at least the following steps: First, in the case of residential radon, the dose is so low that the mean number of α -particle tracks in the target of interest (e.g. the nucleus or a group of cells) is much less than one. In this situation, it follows that a change in the dose simply results in a proportionate linear change in the number of targets struck by a single α particle. Second, it is assumed that cancer induction is causally related to radiation-induced damage in a single cell (i.e., cancers are of monoclonal origin). Consequently, if a single α -particle traversal can produce, with finite probability, the critical damage necessary in a cell to initiate the sequence of events leading to cancer, then the dose–yield relationship will be linear at low doses for production of the critical damage necessary in a cell to initiate the sequence of events leading to cancer, and the dose–response relationship for induction of cancer by low-dose radiation will be linear with dose, and consequently without a threshold in dose.

Of course, the steps in the argument contain uncertainties; for example, it presupposes that an organ with, say, one cell containing critical damage is n times less likely to show a cancer than if it had n critically damaged cells. Is this correct? There is no current evidence to the contrary. The argument also presupposes a monoclonal origin of cancer; the use of approaches involving molecular genetics for the study of the monoclonality of tumors has strongly complemented the traditional approaches to this question, and the evidence that the great majority of cancers are of monoclonal origin is becoming increasingly strong. Of course, by the time a tumor is detected, it will have undergone extensive genetic changes, and so some selection might have occurred, and assessment of clonality at that time might not then reflect the earliest events in carcinogenesis.

Nevertheless, the arguments mentioned above were (and are) quite convincing, particularly at high LET, where a single α -particle traversal through a cell nucleus can clearly produce major genetic disruption. Based on such arguments, and the fact that there currently appear to be no convincing arguments against linearity which would apply at low doses of high-LET radiation, the BEIR VI risk model does incorporate a linear no-threshold assumption. However, the existence of a reasonable underlying biophysical rationale adds significantly to the credibility of the model and its predictions.

Conclusions

In conclusion, both in radiobiology and in radiation epidemiology, it appears that purely phenomenological or statistical approaches to dose–effect relationships have gone about as far as they can go. The BEIR VI report is one of the first examples where insights into mechanisms have been applied to radiation risk estimation.

In the future, it seems likely that identification of damage pathways on the molecular level will be increasingly important. However, qualitative molecular investigations, despite their current popularity, are not likely to be very useful either. The question is not whether a given gene product has some effect or shows some response to radiation; the question is what damage pathways are *dominant* for the important biological end points. This is the current challenge of molecular radiobiology.

From the epidemiological standpoint, with increasing focus on lower doses, input from radiobiology is becoming more and more important. We are still a long way from the day when a grand unified biologically based model of radiation-induced cancer will be of practical use, so it is important that radiation epidemiologists identify areas of major uncertainty, to allow radiobiologists to focus their attentions appropriately, and to use input from radiobiology in those specific areas where it is needed.

Acknowledgments

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Links between Radiobiology and Epidemiology at the Radiation Effects Research Foundation (RERF)

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The explosive growth in understanding of molecular biology and molecular genetics is leading to an increasing emphasis on interactions between molecular biologists and epidemiologists in studies of disease in human populations. Such interactions are especially important in studies of radiation effects on cancer (and possibly other diseases) where they have the potential to provide useful information on the mechanisms by which radiation-induced damage leads to increased risks of disease decades after exposure. As noted by Brenner *et al.* (1), such interactions involve both radiobiological and theoretical considerations in the development of models and the use of information from epidemiological studies to suggest fruitful areas for basic research in radiation biology.

Much of our understanding of the long-term effects of radiation exposure on human health is derived from the epidemiological studies that were begun more than 50 years ago by the Atomic Bomb Casualty Commission (ABCC) and are continued by its successor, the Radiation Effects Research Foundation (RERF). Since the late 1960s ABCC and RERF researchers have studied chromosome aberrations as a biomarker of exposure. This work is continuing in the Department of Genetics along with electron spin resonance measurements of tooth enamel as a biophysical dosimeter. However, it was not until the early 1980s that a radiobiology group was formally established at RERF. Initially the primary focus of radiobiological research at RERF concerned studies of immunological changes and of somatic mutations (such as *HPRT*, glycophorin A and T-cell receptors) that might serve as useful markers of radiation exposure. However, in recent years the emphasis in radiobiological research at RERF has been shifting toward molecular studies of DNA changes that might be directly associated with both cancer development and radiation exposure (e.g. so-called radiation fingerprints or, to a lesser extent, evidence of genetic instability). This changing emphasis is leading to more direct links between epidemiological and radiobiological studies at RERF. As outlined below, because the Foundation has, or has access to, a large quantity of stored biological materials on a fixed cohort of survivors, subject to comprehensive and continuing mortality and cancer morbidity follow-up, we are uniquely positioned to develop and pursue molecular epidemiology studies. The importance of an enhanced role for radiobiology and more interaction between radiobiologists and epidemiologists at RERF was emphasized in the recent multinational review of RERF activities (2) and, more recently, both in a workshop on genetic susceptibility (3) and in an international peer review of research activities and directions in the Department of Radiobiology.

Epidemiology and Related Programs at ABCC and RERF

Concerns about possible genetic effects in the children of survivors of the atomic bombings of Hiroshima and Nagasaki were a major factor leading to the establishment of the Atomic Bomb Casualty Commission

(ABCC). However, since the establishment of the Life Span Study (LSS) cohort in the late 1950s, cohort-based epidemiological studies have been the core of ABCC/RERF research. The primary studies are based on LSS mortality follow-up (4) supplemented with data from a series of periodic mail surveys and studies of cancer incidence (5, 6) using data from the Hiroshima and Nagasaki tumor registries that were established in the late 1950s.

The ABCC/RERF epidemiology studies are supplemented by an ongoing large-scale clinical follow-up (known as the Adult Health Study or AHS) that was begun in 1958 and an autopsy-based pathology program that continued from the early 1960s through the early 1980s. In 1973, the autopsy program was superseded by a tissue registry that records the nature and storage location of histological specimens from residents of Hiroshima and Nagasaki.

Until recently the epidemiology program at RERF has been pursued without much direct consideration of radiobiological issues other than occasional efforts to use "radiobiological" models (e.g. linear-quadratic-linear) models to describe the joint effects of γ -ray and neutron exposures on cancer risks in the LSS. Researchers at RERF and elsewhere are starting to use biologically based models for radiation carcinogenesis, but to date this is a largely theoretical/statistical exercise with little real input from or feedback to radiobiological investigations.

In recent years, we have begun a series of site-specific incidence studies [including studies of tumors of the salivary gland, skin, central nervous system (CNS), liver, lung, lymphatic system and ovaries] based on detailed pathology reviews of relevant materials. These studies are providing a basis for strengthened connections between the epidemiology and radiobiology at RERF. Efforts are also under way to develop a database of partial pedigrees for the RERF cohorts to facilitate molecular and genetic studies in the survivor population.

Recent Activities in Radiobiology at RERF

The focus in the Department of Radiobiology is shifting toward molecular studies involving direct collaboration between radiobiologists and epidemiologists. Recently, RERF researchers have demonstrated that PCR methods can be used to amplify DNA (7) and RNA (8) from archival tissues, making it possible to use historical materials in RERF studies. The initial RERF molecular studies involved a search for evidence of *TP53* (formerly known as *p53*) mutations in LSS lung cancer cases (9). More recently an association between *TP53* point mutations and dose has been found for liver cancer cases in the LSS (10). However, because these are point mutations rather than large deletions and because alteration of *TP53* is likely to be a relatively late event in the development of liver cancer, it seems likely that the changes reflect an indirect effect of exposure. Other molecular studies in progress at RERF include an investigation of activation of the *RET* oncogene in thyroid cancer (including comparisons with thyroid cancer cases among children living in areas affected by the Chernobyl accident), an investigation of the patched gene (*PTCH*) in skin cancer cases, and the development of immunological methods for the characterization of AT heterozygotes.

The department is now working with the Department of Epidemiology to develop a study of early-onset breast cancer cases. This study is a direct outgrowth of the epidemiological observation that the excess risk of early-onset breast cancer appears to be especially high among young women with high doses (11).

Biological Materials

A unique and important aspect of the RERF studies is the availability of biological materials including some samples (for cases and non-cases) collected in the AHS clinical program prior to the diagnosis of cancer for many members of the RERF cohorts. In addition to the tissue samples noted earlier, RERF has, since 1969, collected and stored frozen serum (for ~16,000 AHS participants) and plasma (for ~5,000 AHS participants) samples. Since 1990 lymphocyte samples have been obtained from almost 6,800 people and frozen. Over the past decade immortalized lymphocyte cultures have been created for almost 1,000 LSS members (and

in many cases for their children). There are plans to extend this to most of the remaining clinical study participants. We have also begun to store freeze-dried whole blood on paper disks.

The Departments of Radiobiology, Epidemiology and Clinical Studies are also working with the local medical community to establish new and more effective mechanisms for the collection, storage and use of tissue samples by researchers at RERF and other local research institutions.

Issues

RERF laboratory research is now being focused on studies that integrate radiobiological and epidemiological efforts to provide insight into more general molecular (and genetic) aspects of carcinogenesis. However, because the Foundation's resources are limited and we are, to some extent, constrained by the nature of our mission, we are focusing our efforts on a fairly narrow range of radiobiological studies and seeking expanded collaborations on others. Given the rapid development of molecular biology techniques, what seems experimentally unfeasible today may well be standard procedure in a few years. Thus it is also important for RERF researchers to maintain the flexibility to take advantage of new methods and new ideas about the process of (radiation) carcinogenesis.

Since the understanding of the molecular biology of cancer in general and radiation-related carcinogenesis in particular is still at a fairly early stage in its development, it is essential that our efforts be motivated by reasonable hypotheses and focus on areas in which there is a reasonable chance of gaining important information related to specific hypotheses. The results of epidemiological studies are important in determining where it might be useful to look, especially since the number of high-dose survivors is relatively small and the proportion of radiation-associated cases is not (usually) large. The breast cancer study mentioned earlier is an example in which RERF's epidemiological (and clinical) findings together with recent developments in molecular oncology and genetics suggest feasible and interesting research projects.

The high excess relative risks for thyroid cancers and certain types of CNS tumors among people exposed as children indicate that these areas might also be good candidates for molecular studies. Ideas for molecular studies to be carried out at RERF need not come only from our own research. In particular, the findings of molecular or epidemiological studies in other radiation-exposed populations (particularly radiotherapy patients) have the potential to be a useful source of ideas for specific molecular studies at RERF. In general, we need to develop criteria for selecting end points and hypotheses that can be studied effectively using RERF's valuable but limited resources.

Another area of increasing importance and concern at RERF (as elsewhere) involves ethical issues related to the collection, storage and use of biological materials. RERF staff are working to maintain high standards and to avoid problems that might jeopardize future work.

Over the past five decades ABCC and RERF studies have made important contributions to the characterization and quantification of the long-term health effects of radiation. As understanding of the molecular aspects of carcinogenesis deepens, the unique resources developed by the Foundation are becoming increasingly valuable in understanding the relationship between radiation exposure and cancer. Close ties between radiobiology, genetics, clinical studies and epidemiology are essential if the full potential of this important research is to be realized. At RERF we are working toward this goal.

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DISCUSSION: Can Epidemiologists and Radiobiologists Help Each Other?

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Cancer risk assessment models have evolved considerably since the early work on multistage models in the 1950s by Armitage and Doll. The multistage model is based on the notion that malignant transformation requires that a single somatic cell undergo a finite number of genetic alterations, comprising the stages of carcinogenesis. Although the multistage model is capable of describing the age dependence of most human cancers, it did not take into account other important nongenetic factors in carcinogenesis, such as tissue growth and cell kinetics. These factors were included in the two-stage clonal expansion model developed subsequently by Moolgavkar and his colleagues in the 1970s. Historically, initiation/promotion studies by Berenblum and Shubik (1) provided early experimental motivation for the inclusion of cell proliferation in cancer risk models.

Although these models take into account critical aspects of the process of carcinogenesis, additional factors such as the kinetics of malignant cells, influenced by genomic instability, also require consideration. Denes and Krewski (2) have provided the requisite mathematics to incorporate stochastic stem cell growth in the two-stage model, whereas Zheng *et al.* (3) incorporate DNA repair in multistage cancer models. Other relevant considerations in biologically based cancer modeling include genetic susceptibility and gene–environment interactions, interindividual variability in susceptibility, hormonally mediated effects, and immune suppression.

Considerable experience has now accumulated with the application of cancer risk models (cf. ref. 4). Biologically based models can be fitted directly to epidemiological data, as done by Moolgavkar *et al.* (5) in describing the joint action of radon and tobacco smoke in the induction of lung cancer in Colorado uranium miners. With this approach, it is important to examine the biological plausibility of the estimates of mutation and cell proliferation rates derived from data on lung cancer mortality. This represents an opportunity for epidemiologists and radiobiologists to work together on refining biologically based cancer models, and for supplementing epidemiological data with laboratory data in fitting models.

Epidemiology and radiobiology also intersect in predicting cancer risks in humans. In general, extrapolation of results obtained in nonhuman test systems to humans is needed to support preventive risk management practices. Laboratory data are also useful in elucidating mechanisms of carcinogenic action, in developing and validating biologically based risk models, and in interpreting ambiguous epidemiological data.

Guidance in species extrapolation of radiation risks can be drawn from experience with chemical carcinogens. Although quantitative species extrapolation was traditionally done on the basis of either body surface area or body weight, Travis and White (6) showed that an intermediate scale, body weight to the $\frac{3}{4}$ power, was most consistent with animal and human data. Ames and Gold (7) have questioned the relevance of rodent carcinogenesis bioassays conducted at high doses for human risk assessment, arguing that cellular proliferation observed at high doses may not occur at low doses corresponding to human exposure levels. Using meta-analysis techniques applied to over 400 cancer bioassays conducted under the U.S. National Toxicology Program, Crump *et al.* (8) have provided evidence of additional high-dose carcinogens that may have gone undetected in study-specific evaluations of the data, further compounding this problem. Physiologically based models of pharmacokinetics for determining the dose of reactive chemical metabolites to target tissues (cf. ref. 9) may also be relevant for radiation risk assessment.

The relative biological effectiveness of radiation in different tissues is somewhat analogous to the use of toxicity equivalence factors in chemical carcinogenesis: Both are subject to uncertainty and species variability and should be used with caution (10). A Joint Working Group established by Health Canada and the Atomic Energy Control Board of Canada (11) recently completed a detailed review of methodologies for chemical and radiation risk assessment.

Although practical risk assessment applications tend to rely on empirical risk models such as the linear-quadratic model often used to describe the exposure–response relationship for radiation carcinogenesis, biologically based models are also of value in radiation risk assessment (cf. 5). The BEIR VI Committee opted for an intermediate approach, using radiobiological knowledge about the interaction between α particles and pulmonary tissue to inform model development (12). Although empirical in form, the Committee's two preferred models incorporated both the linear no-threshold hypothesis and dissipation of the inverse dose-rate effect at low levels of exposure. Such interaction between epidemiology and radiobiology is likely to increase, particularly in institutions like the Radiation Effects Research Foundation that house world-class expertise in both fields.

Looking to the future, increased knowledge of cancer mechanisms will provide a stronger basis for biologically based risk models. Model development and validation will be an incremental iterative process, making greater use of molecular and genetic data. Such models will provide greater insight into both interspecies extrapolation and low-dose risk assessment, addressing adaptive responses to radiation at low doses and U-shaped dose–response curves observed with agents like dioxin. Advances in molecular measurement techniques, such as those recently developed by Le *et al.* (13) to measure the formation of DNA adducts at levels several orders of magnitude lower than previously possible, also offer promise as a tool for obtaining more reliable estimates of low-dose cancer risks for both chemicals and radiation.

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II. RADIOSENSITIVE SUBPOPULATIONS: SIGNIFICANCE FOR RISK ESTIMATION AND RADIATION PROTECTION

Chair: Robert Rinsky, *National Institute for Occupational Safety and Health*

Low Doses of Ionizing Radiation and Breast Cancer in Ataxia Telangiectasia Heterozygotes

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The ataxia telangiectasia gene (*ATM*) was recognized initially because it causes a distinctive autosomal recessive syndrome in homozygotes. The excess risk of cancer for AT homozygotes was obvious within a few years after the syndrome was first described by Boder and Sedgewick in 1957 (1).

It became apparent in the 1960s that the *ATM* gene was associated with extreme sensitivity to ionizing radiation. AT patients with cancer suffered devastating necrosis of normal tissues when their cancers were treated with conventional doses of radiation (2). In the 1970s cultured cells from AT patients were shown to be manyfold more sensitive than control cells to killing by ionizing radiation (3). AT cells also have distinctive abnormalities in postirradiation DNA processing. Thus the *ATM* gene is an important gene predisposing to cancer and radiation sensitivity.

The public health impact of mutations at the *ATM* locus became evident in the 1970s when our group showed that AT heterozygotes also have an excess risk of cancer (4), although of smaller magnitude than that of the homozygotes. A much larger retrospective (5) and prospective (6) study of AT families confirmed the excess cancer risk of AT heterozygotes and showed, for the first time, that female AT heterozygotes were predisposed to breast cancer.

It was natural to ask whether ionizing radiation played a role in the cancer excess we observed, because it is the established environmental cause of breast cancer and because the *ATM* gene is associated with

excess radiation sensitivity. Further, cultured cells from AT heterozygotes had shown a modest but significant excess cell killing by ionizing radiation under some specific experimental conditions, especially chronic exposure (7).

The first evidence that some medical diagnostic X rays might increase the risk of breast cancer for women who are AT heterozygotes came from the retrospective family study published in 1987 (5). We observed that 4 of 26 women with breast cancer had had myelograms—a procedure often associated with prolonged X-ray exposure—while only 5 of 288 controls had the same procedure (OR = 10.3; $P = 0.004$).

The 1991 prospective study of cancer incidence (6) further supported the hypothesis that substantial medical X rays predispose AT heterozygotes to breast cancer. Previous exposures were determined from hospital records blindly for the 19 women who had a first breast cancer during the observation period and for 57 matched controls. Subjects were dichotomized into "exposed" if they had at least one fluoroscopic examination of the thorax, radiation treatment or occupational exposure, and otherwise "unexposed".

Ten of the 19 breast cancer cases, and 11 of the 57 controls, were counted as exposed. The odds ratio was 5.8 and $P = 0.005$. The odds ratio may be an underestimate of the relative risk for female AT heterozygotes, since the cases may have included non-carrier blood relatives.

These previous analyses were based on the hospital records collected in the course of each family study. Information about outpatient X rays, including mammograms, was not obtained. Our current study is based on questionnaires about environmental exposures sent to 15 living breast cancer patients and 45 matched controls, all of whom are AT heterozygotes. Exposures to diagnostic X rays or therapeutic radiation were documented from medical records whenever they could be obtained.

Preliminary analysis of the data shows that the most highly exposed group—those with mammograms before 1980 or those who had a CAT scan of the thorax—had a substantially elevated risk of developing breast cancer.

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Cancer after Radiotherapy for Hereditary Retinoblastoma: Genetic Susceptibility and Radiation Exposure

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Retinoblastoma (Rb), a rare eye cancer which accounts for 2.5% of childhood cancers, is considered to be the prototypic hereditary cancer in humans (1). Retinoblastoma occurs in the hereditary form which is due to a germline mutation and subsequent acquired somatic mutation in the *RBI* gene and the sporadic form in which the *RBI* gene acquires two

somatic mutations. The *RBI* gene was the first of the tumor suppressor genes to be identified and associated with a specific chromosomal site, 13q14. Children treated for hereditary retinoblastoma are at exceptionally high risk of second cancers attributed to a germline mutation in the *RBI* gene (2). In particular, sarcomas occur excessively after treatment for hereditary Rb in part due to a shared genetic locus for both diseases. Radiotherapy appears to increase this risk further by causing somatic mutations predisposing to cancer. Retinoblastoma provides an excellent model in which to investigate the relationship between genetic susceptibility to cancer and exposure to ionizing radiation.

To investigate the influence of genetic predisposition in the development of second cancers among survivors of childhood retinoblastoma and to quantify the contribution of radiotherapy to the excess risk of these tumors, in particular sarcomas, we conducted a cohort study of cancer incidence in 1,604 Rb patients (3). We reconstructed radiation doses to sites where sarcomas occurred and performed a nested case-control study to estimate the dose-response relationship for sarcomas after treatment for Rb.

The cohort of 1-year Rb survivors, diagnosed from 1914 to 1984 at two medical centers in New York and Boston, were followed for a median of 20 years after diagnosis. Data on medical history, family history of retinoblastoma, treatment, and second cancers were collected from medical records, radiotherapy records, and two subsequent telephone interviews in 1987 and 1993. Observed numbers of cancers were compared with the expected number of cancers estimated by multiplying person years at risk by age-, sex- and calendar year-specific cancer rates from the Connecticut Tumor Registry. The Kaplan-Meier method was used to estimate the cumulative incidence of second cancers.

Of the 1,604 Rb patients, 961 (60%) had the hereditary form of the disease, 1150 (72%) were diagnosed with Rb before 2 years of age, and 1162 (72%) were alive at the end of follow-up. Patients were determined to have the hereditary form of the disease if both eyes were affected (95%) or if one eye was affected and there was a family history of Rb. Of the 199 second cancers diagnosed, the majority ($n = 190$) occurred among the hereditary Rb patients. We noted an overall SIR of 30 among hereditary Rb patients compared with an SIR of 1.6 in the non-hereditary Rb patients. The predominant second cancers among Rb hereditary patients were sarcomas. No sarcomas occurred in the sporadic Rb patients. SIRs exceeding 100 were noted for cancers of the bone, connective tissue, nasal cavities, eye and orbit, and pineoblastoma (trilateral retinoblastoma) among the hereditary Rb patients. Melanoma occurred in the hereditary Rb patients only (SIR = 51). Brain cancer (SIR = 14) and Hodgkin's disease (SIR = 5.4) were increased in hereditary Rb patients, whereas breast cancer was elevated in the non-hereditary Rb patients (SIR = 5). By 50 years of age, the cumulative incidence of second cancers among hereditary Rb patients was 51% compared to only 5% for non-hereditary Rb patients.

The majority of hereditary Rb patients were treated with radiotherapy (88%) compared with only 18% of non-hereditary Rb patients. Orthovoltage radiation was used to treat Rb patients before 1960, and since then most radiation treatments have been by cobalt-60 teletherapy, betatron or other megavoltage machines. Patients treated after 1980 received electron-beam therapy. Among the hereditary patients, we noted a significant 36.7-fold risk for second cancers after radiotherapy compared with a lower but significant 7.3-fold risk for patients who did not receive radiotherapy. By the age of 50 years in the hereditary patients, the cumulative incidence of second cancers exceeded 58% in the irradiated patients compared with 26% in the nonirradiated patients. Among non-hereditary Rb patients, there was a 2.7-fold risk for second cancers among irradiated patients compared with a 1.3-fold risk for nonirradiated patients.

We performed a nested case-control radiation dosimetry study of sarcomas after treatment for retinoblastoma. There were 63 bone sarcoma cases and 37 soft-tissue sarcoma cases, all of which occurred in hereditary Rb patients. We randomly selected 100 hereditary Rb control subjects who did not develop a second cancer. We estimated radiation dose to the site of the sarcoma for each case, and dose to every anatomical site at

which sarcomas occurred in the cases for each control. Mean doses to the bone tumor sites were 32 Gy for cases and 20 Gy for controls, whereas the mean doses were 20 Gy to the soft-tissue sites and 10 Gy for controls. Compared with the referent dose of <5 Gy for soft-tissue sarcomas, the odds ratios rose with increasing dose up to 11.7 at 60 Gy. For all bone and soft-tissue sarcomas combined, a similar pattern was seen. We estimated a 16.6% increase (95% CI 2.5%–1630%) in RR per gray for soft tissue sarcomas and a 19% increase in RR per gray (95% CI 14%–31%) for all sarcomas combined.

This is the largest series of Rb patients studied for the occurrence of second cancers, and this cohort also included a greater proportion of hereditary compared with sporadic Rb patients than occurs in the general population. Therefore, this cohort provides an enriched population in which to investigate the effects of genetic predisposition to cancer and its relationship to ionizing radiation. We found in our study that hereditary patients by the age of 40 had exceeded the lifetime cancer risk in the general population. Genetic predisposition appears to have a large impact on the risk of second cancers, given the huge disparity in risks of second cancer between the hereditary and non-hereditary Rb patients. Radiation appears to enhance this risk, further suggesting a possible gene-radiation interaction. A radiation dose response has been reported previously for bone sarcomas after treatment for Rb (4, 5), but this is the first report of a dose response for soft-tissue cancers in humans.

We have recently expanded the Rb cohort and plan to continue follow-up of this valuable population to determine whether their cancer risk extends into later life and whether the same or different types of tumors will develop in later adulthood. We also plan to perform a pooled analysis of osteosarcomas after treatment for Rb with data from two other studies. Blood samples from a subset of our Rb patients, primarily hereditary patients with second cancers, have been collected to identify specific mutations in the *RBI* gene that may be associated with an increased risk of second cancer in these patients.

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Incorporating Radiosensitive Subpopulations into Radiation Risk Estimates

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Radiation risk estimates for genetic and carcinogenetic effects have typically considered average populations as far as sensitivity and suscep-

ability are concerned since the databases used have not adequately allowed for a departure from this. The relative radiation sensitivity of the human populations or laboratory animals used for developing risk assessments has been addressed, but in a theoretical rather than an experimental way. Recent advances in our knowledge of the mechanisms underlying the development of cancer and inherited genetic diseases has allowed for a reconsideration of the relevance of radiosensitive subpopulations to the risk assessment process (1, 2).

It has been firmly established that cancer is a genetic disease (3), generally requiring several mutations to move a normal cell along the multistep pathway to a tumor. It has also been determined that alterations in oncogenes or tumor suppressor genes are the most frequent genetic alterations involved in tumor development. In addition, mutations in DNA housekeeping genes, such as those involved in DNA repair or replication, are indirectly involved in the process by increasing the mutation rate for genes whose alteration is essential for tumor development, i.e. mutator phenotypes. The genetic basis for cancer means that there is a significant opportunity for there to be susceptible individuals who are heterozygous at loci involved in the cancer process directly or indirectly via mutator phenotypes. This phenomenon is the basis for Knudson's two-hit hypothesis for explaining the development of bilateral (inherited mutation) compared to unilateral (somatic mutation) retinoblastoma (4). This susceptibility has been clearly demonstrated for a number of syndromes associated with heterozygosity for tumor suppressor genes, e.g. Li-Fraumeni (*TP53*^{+/−}), early-onset breast cancer (*BRCA1* or *2*^{+/−}) and von Hippel Lindau (*VHL*). The same syndromes can be used to illustrate how germinal effects can alter susceptibility and be incorporated into genetic risk assessment. For example, a mutation in a tumor suppressor gene in a germ cell leading to heterozygosity will increase subsequent susceptibility to cancer in any heterozygous offspring. This will result in a combination of genetic risk and cancer risk.

The question of particular relevance to the present discussion is how radiation exposure and radiation sensitivity to mutation induction, for example, among different genotypes have an impact upon cancer risk assessments for the whole population and for susceptible subgroups. The primary issue is to identify radiation-sensitive subgroups, which despite fairly extensive research has proven to be quite elusive (5). The quintessential example of individuals susceptible to cancer and sensitive to radiation-induced mutation are those with ataxia telangiectasia, being homozygous for the recessive *ATM* gene. The relative radiation sensitivity of the *AT* heterozygote to cancer induction is much less clear-cut. For purposes of risk assessment, knowledge of effects in heterozygotes is much more pertinent, given that in the great majority of cases the frequency of the heterozygote is much greater than the homozygous recessive, for rare recessive genes. In addition, it remains of considerable importance to consider the impact of exposure to any given susceptible subpopulation within the context of the range of risk within the whole population.

The identification of relative risk to radiation exposure has been extended to other subpopulations heterozygous for a specific tumor suppressor. Limited evidence suggests an increased cancer risk for radiation exposures in individuals heterozygous for the *TP53* gene (Li-Fraumeni), *RBI* gene (retinoblastoma) and *NBCCS* gene (nevroid basal cell carcinoma syndrome). Other ongoing studies are investigating possible relationships between *BRCA1* and *BRCA2* (breast cancer) and *NF1* (neurofibromatosis) and sensitivity to radiation-induced cancer. Recent evidence has indicated relationships between tumor suppressor genes and DNA repair and cell cycle control; mutations in such genes would lead to increases in radiation-induced mutagenicity. The extent of any increases in sensitivity to cancer will help guide the inclusion of such subpopulations into risk assessment models.

An additional component to the discussion is how sensitivity is related to dose; namely, what are the differences for cancer-susceptible individuals at low environmental exposures, where the impact of small increases in sensitivity in large population groups would have great significance for risk assessment. Very little is known that could support an answer. In a similar vein, rather little is known about sensitivity to chronic expo-

sure, since most of the studies linking tumor suppressor gene heterozygotes with sensitivity for radiation-induced cancer have been for analysis after radiotherapy. The development of research plans for the future is relatively clear-cut; methods are rapidly becoming available for addressing the pertinent questions.

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III. INDOOR RADON AND RISK OF LUNG CANCER

Chair: Ernest G. Letourneau, *Health Canada*

Iowa Radon Lung Cancer Study

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Introduction

To assess the association between radon exposure and lung cancer, we conducted a population-based case-control study of Iowa women aged 40 to 84 who had lived in their current home for at least 20 years. Iowa is an excellent location to perform such a study for several reasons: (1) A substantial proportion of Iowa's population resides in the same home for 20 years or more; (2) Iowa has a high-quality, National Cancer Institute-supported Surveillance, Epidemiology, and End Results (SEER) registry for cancer reporting, which allows rapid identification of newly diagnosed lung cancer cases; and (3) Iowa homes contain the highest mean screening ²²²Rn concentrations in the United States. The Iowa Radon Lung Cancer Study's (IRLCS) unique combination of study design, enhanced dosimetric techniques, individual mobility assessment, population stability, expert histopathological review, and high percentage of live cases provided a rare opportunity to investigate whether or not residential radon exposure exhibits a statistically significant positive association with lung cancer.

Methodology

The IRLCS has four major components: (1) rapid reporting of cases, (2) a mailed questionnaire followed by a face-to-face review and facilitated interview, (3) a comprehensive ²²²Rn exposure assessment, and (4) independent histopathological review of lung cancer tissues (1).

Case subjects. Lung cancer cases met the following inclusion criteria: (1) newly diagnosed with a primary invasive (not *in situ*) lung carcinoma, without any prior primary invasive lung carcinoma; (2) female Iowa resident at time of diagnosis; (3) age ranging from 40 to 84; (4) primary lung carcinoma confirmed microscopically; and (5) residence for 20 consecutive years or more in the current home. Cases that met the eligibility

criteria were identified by the Iowa Cancer Registry (ICR) between May 1, 1993, and October 30, 1996. Sixty-nine percent of the case subjects were alive at the time of the interviews.

Control subjects. Control subjects met the following eligibility criteria: (1) no prior primary invasive lung carcinoma at the time of initial contact as determined by the ICR database; (2) female Iowa resident at time of initial contact; (3) age ranging from 40 to 84; (4) alive at time of interviews; and (5) residence for 20 consecutive years or more in the current home. Control subjects aged 40–64 were selected from current driver's license tapes provided by the Iowa Department of Transportation. Control subjects aged 65–84 were selected from a current tape made available through the Health Care Financing Administration (HCFA). These two databases were chosen to provide a population-based sampling frame. Both driver's license and HCFA controls were frequency matched by age using 5-year age groups with the lung cancer cases.

Radon dosimetry. The radon dosimetry assessment consisted of five components: (1) on-site residential assessment survey, (2) on-site radon measurements, (3) regional outdoor radon measurements, (4) assessment of subjects' exposure when in another building, and (5) linkage of historic subject mobility with residential, outdoor and other building radon concentrations. Component 1 was a residential assessment, and dosimetry placement, which was conducted in person at the subject's home. Upon arrival at the home, the field technician reviewed the mailed household questionnaire with the participant to check for missing or inconsistent information. Next, a home survey was performed that documented home characteristics, location of rooms and dimensions, number of home levels, and environmental γ -radiation levels. During the home survey, the field technician recorded home floor plans, room location of detector placement, detector placement location within a room, house level of placement, time and date of placement, and detector control numbers.

A major part of the residential assessment included a mobility review. Historical participant mobility within the home as well as time spent outside the home and in another building was ascertained by a face-to-face interview with the study participant. Beginning with the year the participant moved into the current home, the interviewer prompted the participant to go forward in time and identify periods where their mobility patterns remained relatively stable. Within these temporally stable periods, hours spent in another building, outside and within the home were collected using task linkage (e.g. retrieval of hours based on time spent involved in specific duties or activities). Each participant-reported period was identified using autobiographical memory cues and facilitated using task linkage. Using this methodology, all time (168 h per week) was accounted for from the year they moved into their current home to enrollment in the study (1, 2).

The second component of the radon dosimetry assessment was on-site measurement of home radon gas concentrations for each case and control (1). Using the results of the mobility interview, the field technician placed radon detectors in rooms where the subject spent most of her time either the year prior to diagnosis for cases or the year prior to initial contact for controls. At least one monitor was placed on each level of the home, in current and historic bedrooms, and in home work areas (1, 3). Landauer's Radtrack® Alpha Track Detector (ATD) was used to provide an integrated mean concentration of residential radon gas. The radon detectors remained within the participants' home for 1 year, after which time a field technician retrieved the detectors, noting any improper movement of the detectors. The IRLCS followed a strict Quality Assurance (QA) plan for proper placement and removal of radon detectors, which included telephone contacts with study subjects quarterly during the year-long dosimetry period to ensure that the radon detectors remained appropriately placed (4). The dosimetry QA portion of the plan was guided by Environmental Protection Agency Guidelines. Three components were used to monitor detector performance: (1) Five percent of detectors were exposed (spikes) to known quantities of radon to test the accuracy of the detector's response; (2) 12% of the detectors were collocated (duplicates) to monitor the precision of the detectors' response; and (3) 5% of the detectors were unexposed (blanks) to examine whether or not the detectors picked up extraneous exposures. Detectors were exposed to known

radon concentrations in the U.S. Environmental Protection Agency's Eastern Environmental Radiation Facility in Montgomery, AL. Electret Passive Environmental Radon Monitors (E-PERM) detectors were also collocated with ATDs at 2% of the sites as field intercomparison detectors. A termination survey was performed at the end of the monitoring period for each placement to retrieve dosimetry, at which time the participant, or next of kin, completed a final questionnaire that ascertained information on changes in home construction or behaviors that may have affected radon concentrations during the monitoring period. Movement or damage of detectors was recorded. A QA Officer from outside the study periodically reviewed all aspects of radon measurements, including field procedures, data management, data collection, laboratory correspondence, data analyses, reports and data archives (4).

The third component of the radon dosimetry assessment was the measurement of mean outdoor radon concentrations (5). One hundred eleven geographically dispersed locations in Iowa were measured by 129 U.S. Environmental Protection Agency-proficient α -particle track detectors. The detectors were housed in weatherproof chambers held 1 to 2 m above the ground and placed for 1 year at least 10 m from a home. Side-by-side duplicates, 1- and 2-m pairs, and year-to-year pairs suggested instrumental, vertical and annual variations of less than 15% (3.7 mBq/liter). Outdoor radon concentration contour maps were generated from the outdoor radon measurements by variogram and kriging analyses. The data's spatial distribution was analyzed through variogram modeling to determine the best functional representation and correlation vector for the complete data set. This modeling was used to construct a surface map of outdoor radon concentrations through a procedure known as kriging. Each point on the kriged surface map was a weighted local average of the direct outdoor measurements. Outdoor radon exposure was estimated using this surface map. The exposure model assumed that a subject is exposed to outdoor radon within a 20-mile radius of her home.

The fourth component of the radon dosimetry assessment was the estimation of radon concentrations for each subject in other buildings (workplace, church, store, etc.). The distribution of radon concentrations in workplaces is not well documented. We have studied the relationship between bedroom radon and workplace radon for approximately 100 women in nearby Minnesota. The results from this study suggest that the best estimate for the workplace radon is 0.5 times the bedroom radon concentration. Thus we also constructed a kriged surface map for the other buildings based on 0.5 times the first-floor radon concentrations within the control subjects' homes. This map was then used to estimate other building radon exposures. The exposure model assumed that a subject was exposed to radon in other buildings within a 20-mile radius of her home.

The fifth component of the radon dosimetry assessment was the linkage between the various radon concentrations and both the subject's temporal and spatial mobility (1–3). A time-weighted average radon exposure for each subject was calculated based on average year-long radon measurements performed in the current bedroom (and historic bedroom, if applicable); each level of the home; and in-home work area (if applicable). The average year-long radon measurement was linked to percentage time spent in bedroom (and historic bedroom, if applicable), each level of the home, in-home work area (if applicable), outdoors, in another building, and on vacation for each subject. The current average year-long radon measurement was assumed to be constant over the years the participant lived in the home; however, the temporal and spatial activity (time spent in the bedroom, each level of the home, etc.) was allowed to vary for each subject by individual season and period as recorded in the mobility interview. The mobility interview accounted for 168 h per week allowing for differences between weekdays and weekends, seasons (warm weather and cold weather, etc.) and employment outside of the home or work in the home. Dose assessment included exposure for all years the subject lived in the current home.

Results

Preliminary data indicate that a positive association exists between residential ^{222}Rn gas exposure and lung cancer. Future research includes

analyses of data that have already been collected from historic reconstruction detectors (HRDs). These detectors provide retrospective ^{222}Rn progeny dose estimates by examining implanted ^{222}Rn progeny within glass surfaces.

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Indoor Radon and Lung Cancer Risk: A Case-Control Study in Connecticut and Utah

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Studies of miners have demonstrated a dose-related lung cancer risk from exposure to radon, but residential studies have been inconclusive (1). We carried out a case-control study in Connecticut, Utah and Southern Idaho to assess lung cancer risk associated with lifetime residential exposure to radon among smokers and nonsmokers. Persons aged 40-79 with incident pathologically confirmed lung cancer were identified through cancer registries and medical record review. Based on a screening telephone interview, all persons who had never smoked (never-smokers) or who had not smoked for at least 10 years (nonsmokers) and a random sample of smokers were selected for study. Persons who smoked cigars or pipes but not cigarettes were excluded.

Controls were identified through random telephone screening and listings of Medicare recipients provided by HCFA (for controls aged 65 or older in Utah/Idaho). Controls were selected using randomized recruitment to achieve a sample that was effectively matched on smoking status 10 years prior to interview, age and gender, but without the resulting analytical limitations of matching (2, 3). In general, we chose one control per case. However, two controls per case were selected for never- and nonsmokers in Utah. Additional selection criteria based on duration of adult residence in study states, number of lifetime residences, employment in mining, and ability to complete the interview were imposed.

Telephone and in-home interviews were completed to obtain detailed residential histories as well as information on medical history and other potential lung cancer risk factors. Study subjects provided detailed information about characteristics of each house occupied for at least 1 year. They also provided information on hours spent on various floors of the home, where they slept, and whether or not they worked outside the home. Floors within a home were characterized with respect to their distance from ground level.

A total of 1474 cases (963 in Connecticut and 511 in Utah/Idaho) and 1811 controls (949 in Connecticut and 862 in Utah/Idaho) completed the study. Only 6% of cases in Connecticut and 10% of cases in Utah/Idaho had never smoked. However, only 17% in Connecticut and 23% in Utah/

Idaho were current smokers at the time of diagnosis. Nearly all study subjects were white. Forty-nine percent in Connecticut and 34% in Utah/Idaho were females.

An attempt was made to measure radon in all homes that were occupied by the study subject for at least 1 year since age 25, as well as in the longest childhood residence. Detailed information on house characteristics, including changes that may have been made to the home, was also sought from current occupants of prior residences of study subjects. In all homes, year-long track-etch detectors were placed in the study subject's bedroom, another room on the lowest living level where substantial time was spent, and in the basement (if 1+ h/week spent in the basement). In a sample of the multilevel homes, detectors were placed on every living level. For every 20th detector, a second detector was placed side-by-side for quality control and a third was set aside for controlled exposure (either blank or spiked).

Average radon concentrations were lower than anticipated. Among measured homes, the geometric mean radon concentration on the floor closest to ground level (index level) was 20.72 mBq/liter in Connecticut and 45.51 mBq/liter in Utah/Idaho. Only 3% of homes in Connecticut and 7% of homes in Utah/Idaho exceeded the EPA action level of 148 mBq/liter, but 11% of homes in Connecticut and 25% of homes in Utah/Idaho had index-level radon values that exceeded 74 mBq/liter.

A total of 13,380 unique residences (an average of 4 per participant) were reported during the time window of interest (age 25 up to 5 years prior to diagnosis). Of these, 10,221 (76%) were considered eligible for measurement because they were occupied by the subject for at least 1 year and were not more than two stories above ground level, institutions or mobile homes without a permanent foundation. Radon was measured successfully in 57% of eligible homes in Connecticut and 60% in Utah/Idaho. Nonetheless, measured homes tended to be long-term residences, and coverage (with radon measurements) of the time window of interest is more complete. In Connecticut, 79% of study subjects had at least half of the years in the time window of interest accounted for by one or more radon measurements. The corresponding percentage in Utah/Idaho was 83%. Whereas only 20% of study subjects had complete coverage from age 25, 62% had complete data for the period from 25 to 5 years prior to diagnosis or interview and 78% had complete data for the period from 15 to 5 years prior to diagnosis or interview.

Radon levels for unmeasured floors within a home were estimated from linear regressions based on paired results from similar homes with complete data. For example, using all homes where measurements were obtained for both the first and the second floor, the linear relationship between the first and the second floor was determined to estimate second-floor values from measured first-floor values. Average lifetime exposure to radon (age 25 to 5 years prior to diagnosis) was calculated as a time-weighted average of amount of time in each residence and proportion of time spent on each level of a home.

We used mean values from measured control homes to "impute" radon values for similar homes that could not be measured (4). Regression trees were constructed separately for Connecticut and Utah/Idaho using measured control homes to identify categories of residences that were similar in their radon concentration. Predictors used in constructing the trees included the relative (to ground) position of the index level, housing characteristics, and geological characteristics (e.g. altitude, groundwater radon, soil permeability, atmospheric radiation) obtained by linking geocoded study residences to available geographic databases. An index level radon value for each unmeasured home was imputed from the mean radon value for all measured control homes in the appropriate strata identified from the tree.

Estimates of lung cancer risk associated with cumulative radon exposure and exposure during various periods or at specific ages prior to diagnosis will be reported, taking into account smoking and several measures of socioeconomic status, which appears to be correlated with both case status and the likelihood of obtaining a measured radon level.

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Cumulative Residential Radon Exposure and Risk of Lung Cancer in Missouri: A Population-based Case-Control Study

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Context

All previously published studies of lung cancer risk associated with residential radon exposure have used a radon dosimetry procedure (i.e. either ambient air radon dosimetry, also called Radtrack® detectors or charcoal canisters) which assumes that current measurements of radon in the dwelling are reflective of historical radon levels in that same dwelling (1). Emerging data indicate substantial variation in annual radon concentrations measured in the same dwelling, calling into question the validity of previous epidemiological results where annual residential radon levels varied from year to year.

Objective

To investigate the association reported previously in several studies between residential radon exposure and the risk of lung cancer, we used a new radon monitoring technology which measures cumulative radon exposure directly for the previous 20-30 years (2-6).

Design

This is a population-based case-control study of lung cancer among women. A structured questionnaire was administered by telephone with follow-up questionnaire and residential measurements made in person in the subject's home.

Subjects. All newly diagnosed female patients aged 30-84 years in Missouri from 1 January 1994-31 January 1994 diagnosed with primary lung cancer that was reported to the state cancer registry were invited to participate ($n = 742$) together with population-based controls ($n = 730$). A two-stage randomized recruitment procedure was used to mitigate the extreme imbalance in smoking frequency between cases and controls.

Radon dosimetry. Two year-long ambient air radon measurements were sought in every dwelling occupied by the study subjects in the previous 25 years to estimate cumulative radon exposure indirectly. In addition, using a technique newly applied to epidemiological investigations, CR-39 α -particle detectors (also called surface monitors) were affixed to subjects' selected household glass objects to estimate cumulative residential radon exposure directly.

The main outcomes measured were odds ratios (95% confidence interval) for the development of lung cancer associated with residential radon exposure, by age of onset, cell type and smoking history.

Results

The trend in lung cancer odds ratios did not vary with increasing radon concentration measured by standard track-etch detectors. However, a significant positive dose-response trend was observed with radon measurements from surface monitors.

Conclusions

A significant dose-response trend between cumulative radon detector measurements and lung cancer risk was observed at concentrations below the limits currently recommended by the U.S. and European countries.

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Analysis of the Combined Primary Data from Residential Radon Studies in North America: A Status Report

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Radon, a naturally occurring gas released from most rocks and soils, is present at low levels in most North American homes. Because underground miners exposed to high levels of radon have been shown to be at excess risk of lung cancer, concerns have been raised about the potential risk arising from residential exposures (1). Using data on residential radon exposures in the U.S. in conjunction with data on lung cancer mortality from 11 cohorts of underground miners, the National Research Council BEIR VI Committee (2) recently estimated that radon contributes to 10-15% of the approximately 157,400 lung cancer deaths that occur each year in the U.S.

Because of the numerous sources of uncertainty involved in extrapolating risks from such occupational studies to the general population, epidemiological studies have also been undertaken in residential settings. Eight large population-based case-control studies involving direct measurements of residential radon exposure have been completed to date in Canada (Winnipeg), China (Shenyang), Finland (two studies), Sweden (two studies) and the United States (New Jersey and Missouri). Although the results of the individual studies have been somewhat equivocal, a meta-analysis of the summary odds ratios published by the original investigators demonstrated a significant association between residential radon exposure and lung cancer risk that was consistent with the results from the occupational data (3).

An analysis of the combined primary data from a series of similar investigations has several advantages over a meta-analysis of the reported summary level results of the same investigation.¹ Access to the primary data can improve internal and external validity by enabling the analyst to: (1) define common criteria for the eligibility of subjects; (2) analyze the exposure data in the original scale of measurement, using a uniformly defined exposure time window, and a standard method for the imputation

¹ V. S. Catalan, Analysis of the combined primary data from case-control studies of residential radon and lung cancer: A pilot study of three North American sites. Ph.D. Thesis, Department of Epidemiology and Biostatistics, McGill University, Montreal, 1998.

of missing data; and (3) ensure that a common set of *a priori* risk factors and effect modifiers are examined and treated according to consistent analytical criteria.

An analysis of the combined primary data from seven North American case-control studies (Connecticut/Utah/Idaho, Iowa, Missouri I and II, New Jersey I and II, and Winnipeg) was initiated at an international meeting of radon researchers in February 1995 sponsored by the U.S. Department of Energy and the Commission of European Communities (4). The decision to proceed with this analysis at that time followed a number of previous discussions on this initiative. Officials from Health Canada hosted a subsequent planning meeting in October 1995, including the principal investigators for all completed and ongoing North American case-control studies, other invited scientists with expertise in radon risk assessment, and representatives from the U.S. Department of Energy and the Commission of European Communities. At that meeting, all investigators agreed to submit their primary data according to a common format. After a subsequent planning meeting hosted by Health Canada in June 1997, the data available from the three completed North American case-control studies were included in a pilot analysis.¹ The three included studies were Missouri I, New Jersey I and Winnipeg, and involved a total of 1,590 cases and 2,215 controls.

The final data format for the pilot analysis included both static (age, year of case and control ascertainment, source of information, gender, active and passive smoking, education, family income, and ethnicity) and time-dependent variables (home sequence identifier, intensity of active smoking by the study subject and co-residents, radon concentration in the living area and basement, radon estimation method for the living area and basement, and proportion of time spent in the home). Prior to inferential analyses, these data were examined carefully to confirm correspondence with the originally published descriptive statistics. To obtain retrospective radon exposures for the period 5 through 50 years prior to enrollment into the study, missing measurements were imputed using the observed control mean as recommended by Weinberg *et al.* (5). A common logistic regression model involving both *a priori* and empirically chosen covariates was developed through parallel analyses of the three data sets. Modification of the radon effect by smoking, age at ascertainment, and gender was explored. The two-stage random-effects regression methods as described by Wang *et al.* (6) were then employed to derive an estimate of the combined overall radon effect. Sensitivity of the results to the definition of the time window of exposure and form of the statistical model were also investigated.

Preliminary results for three of the four ongoing studies were presented at the 1998 ASA Conference on Radiation and Health, and the extended abstracts appear in this volume (see abstracts by Field *et al.*, p. 101; Sandler *et al.*, p. 103; Alavanja *et al.*, p. 104). The Iowa case-control study includes extensive information on spatial variation of radon levels within residences and on radon exposures occurring outside the home. Restricting study subjects to people who had resided in the same home for at least 20 years minimized the need for imputation of radon values in this study. In addition to using current ambient radon measurements to predict historical radon exposures, both the Iowa study and Missouri II employed CR-39 α -particle track detectors affixed to selected household glass objects to estimate cumulative radon exposure more directly. All three studies included duplicate radon measurements in at least some homes, which will permit an assessment of exposure measurement error. Data from the four remaining studies, representing approximately 3,337 additional cases and 3,928 controls, were expected to be available for a complete analysis by the fall of 1998.

The North American pooling effort will be the largest study conducted to date of residential radon exposure and lung cancer, including some 4,927 cases and 6,143 controls. The available data will permit a more powerful examination of the critical exposure time window and potential modifiers of the association between residential radon and lung cancer than was possible in the pilot analysis, and will include additional factors previously unavailable, such as ventilation habits (sleeping with an open window in Utah/Idaho), activity-weighted particle size distribution, and equilibrium fraction (in Iowa). Following Darby *et al.* (7), attempts will

be made to account for error in radon exposure estimation. It is anticipated that a preliminary analysis of the combined primary data will be the subject of the next meeting of the study participants in 1999.

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DISCUSSION: Indoor Radon and Risk of Lung Cancer

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Introduction

There have been eight case-control studies of residential radon and lung cancer published to date (1). Preliminary results have been presented at this conference on three studies (in Missouri, Iowa and Connecticut/Utah). In addition, results were presented at the 11th Annual Lectures on Radon, sponsored by the Institut für Strahlenschutz and held 18-19 March 1998 at the GSF-Forschungszentrum für Umwelt- und Gesundheit, Neuherberg, by S. Darby on a study in Cornwall and Devon in the United Kingdom (2) and by E. Wichmann on studies in the western and eastern parts of Germany. This brings to 14 the number of studies of indoor radon and lung cancer, with a total of 9,885 cases and 16,539 controls (Table 1). Except for the Cornwall/Devon study, the newer studies have not yet been published in peer-reviewed journals, and thus results should be considered preliminary.

The Connecticut/Utah study was presented at the ASA conference, but results were considered incomplete and are not included in Table 1 or in the following calculations.

Improvements in Study Design

The earliest studies of residential radon and lung cancer were targets of opportunity, with radon measurement protocols for houses added to ongoing lung cancer case-control studies. While the addition of measurement protocols to existing studies was not inherently limiting and did not necessarily result in biased estimates of relative risk (RR), the studies were not designed specifically to assess residential radon and so may have had limited coverage of the exposure time window, or may have

TABLE 1
Relative Risks (RR) and 95% Confidence Interval (CI) for Residential Radon Case-Control Studies

Study	RR ^a	95% CI	Cases	Controls
Finland I	1.30	1.1-1.6	164	334
Finland II ^b	1.26	1.1-1.6	517	517
New Jersey, U.S.	1.83	1.2-2.9	433	402
Shenyang, China	0.84	0.8-0.9	308	356
Winnipeg, Canada	0.96	0.9-1.1	698	738
Stockholm, Sweden	1.83	1.3-2.5	201	378
Sweden	1.20	1.1-1.3	1,281	2,576
Missouri I, U.S.	1.12	0.9-1.4	538	1,183
Missouri II, U.S.				
Surface ^c	2.29	1.7-3.1	372	471
Track-etch	0.78	0.6-1.0	247	299
Cornwall/Devon, UK	1.19	1.0-1.4	982	3,195
Western Germany ^d				
Total	1.04	0.9-1.3	1,449	2,297
Prone areas	2.66	2.0-3.5	365	595
Eastern Germany				
Total	1.16	1.0-1.4	1,053	1,667
Prone areas	1.40	1.0-1.9	NA ^e	NA
Iowa, U.S.	1.43	1.3-1.6	415	614
Connecticut/Utah	NA	NA	1,474	1,811
Total			9,885	16,539

^a RR at 150 Bq m⁻³ based on a log-linear model, $RR(x) = \exp[\beta(x - x_0)]$, fitted to category-specific, adjusted RRs for each study, where x is the mean radon level and x_0 is the mean of the lowest category.

^b RR estimate and 95% CI based on corrected data in erratum (3).

^c Exposures estimated from CR-39 surface monitors or on track-etch air radon monitors.

^d German results reported for complete data and for data restricted to radon-prone areas.

^e Information not available.

collected fewer data on housing characteristics and other pertinent information. In addition, several of the earliest studies derived exposure estimates from radon measurements of 2 to 3 months duration, rather than 1 year, as in the more recent studies, or used short-term measurements of 1-2 weeks to supplement missing radon concentrations.

In contrast, the recent studies have included design elements that are associated with more complete and accurate radon measurement data and thereby more precise estimates of exposure (Table 2). Some of the important design elements are: more complete assessment of radon exposure by measuring all rooms of the house, as well as measuring outdoor radon levels; minimum residency restrictions for the current house, thereby limiting enrollment to subjects who were long-term residents; and use of surface radon detectors (CR-39 plastic on glass artifacts) that measure (average) radon concentration over many years. Improved study designs likely produced better-quality exposure data, although it cannot be known whether the studies that included some or all elements of Table 2 did in fact have more accurate exposure estimates.

Results of Studies and Summary Estimates from Meta-Analysis

As in Lubin and Boice (1), for each study we fitted the log-linear model, $RR(x) = \exp[\beta(x - x_0)]$, to the category-specific, adjusted RRs, where x is the mean radon level and x_0 is the mean of the lowest category. Table 1 shows estimates from the fitted model for 150 Bq m⁻³, i.e. $\exp(\beta \times 150)$, for the previous eight studies (with the corrected Finland II data) and for the newest indoor radon studies.

For the newest studies, a significant exposure response for indoor radon was found for the Missouri II study when exposure estimates were based on CR-39 surface monitors, which were placed on glass artifacts and measured the long-term (average) radon concentration, but there was

TABLE 2
Improvements in Design of Recent Studies of Residential Radon and Lung Cancer

Restrictions on minimum residency time in current houses
Restrictions on the maximum number of houses lived in
Use of year-long radon detectors
Use of radon detectors that directly measure (average) exposure rates over many years
Increased efforts to measure or estimate radon exposure from all sources (e.g. all rooms of houses, outdoors, workplaces)
More complete information on residential occupancy, time spent indoors and within each room of house
More detailed information on housing characteristics and modifications
Use of unbiased methods for imputing missing radon data
Increased numbers of cases and controls
Increased power for analysis of smoking and radon exposure using randomized recruitment

no trend when exposure estimates were based on standard year-long track-etch detectors. The reason for this difference was unknown and was still being explored. A significant exposure-response relationship was found in the Iowa study.

The three studies presented at the Lectures on Radon in Germany showed mixed results. In the Cornwall/Devon study, RRs increased with increasing residential radon concentration (2). In this study, the investigators included a detailed evaluation of the impact of exposure misclassification on the estimate of trend in risk (4). The excess RR per Bq m⁻³ was increased about 50% after adjustment for measurement error. The studies in Germany were two of the largest studies and results depended on whether or not data were restricted. The exposure-response trends were statistically significant when data were limited to those areas defined as "radon prone". In the study in the eastern part of Germany, the exposure-response trend in the full data set was statistically significant, while the exposure-response trend in the restricted data was notably diminished in magnitude although still statistically significant. In the study in the western part of Germany, there was no significant exposure-response trend in the full data set. The reasons for different results based on data restrictions are as yet unexplained, but may have been related to patterns of exposure error.

Using data from the eight previously published radon studies, the estimated RR at 150 Bq m⁻³ based on log-linear models fitted to each of the studies and then combined into a summary trend was 1.18 with 95% CI (1.0, 1.4), indicating an overall significant risk of lung cancer from indoor radon. This estimate updates the RR estimate of 1.14 given by Lubin and Boice (1), who included the uncorrected Finland data. Based on the studies in Table 1, the summary estimate of the RR at 150 Bq m⁻³, using results from the Missouri II study with exposure based on surface monitors and from the German studies with restricted data, was 1.35 with 95% CI (1.2, 1.5). The RR at 150 Bq m⁻³, using results from the Missouri II study with exposure based on track-etch monitors and from the German studies without data restrictions, was 1.16 with 95% CI (1.0, 1.3).

As demonstrated in the report of the National Research Council's Committee (BEIR VI), the excess risks observed in the residential radon studies are consistent with predicted levels of risk from models developed from data on underground miners exposed to radon (5). In addition, the excess risks in the residential studies are consistent with RRs observed in miners with cumulative exposures similar to exposures experienced by long-term residents in high-radon houses.

Conclusion

The results from newest case-control studies of indoor radon continue to support the existence of a small excess lung cancer risk to the general population from residential radon. This excess is entirely consistent with extrapolations using models developed using data for miners.

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IV. DOSIMETRY AT LOW DOSE RATES

Chair: Richard W. Hornung, *University of Cincinnati*

Accounting for Bias and Measurement Error in Occupational Studies

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This work is motivated by the need to adjust for dose bias and uncertainty in epidemiological dose-response analyses. Typically, epidemiological studies of the effects of external penetrating radiation on worker health have relied on recorded annual doses to the individuals in the population. At Oak Ridge, these annual doses were obtained by adding up recorded weekly readings. In statistical analyses, these dose values have been treated as though they are known exactly, although everyone recognizes that there is uncertainty due to measurement error and bias. It is usually assumed that the measurement errors "average out" and that the bias is small. A recent study of Oak Ridge workers (1) used a preliminary dose adjustment procedure and found an upward bias in dose-response coefficients and likelihood ratio test statistics. This analysis was based on a crude adjustment for missing dose and did not consider measurement and other dosimetry errors.

Although our goal is to account for bias and uncertainty in occupational risk estimates for ionizing radiation, we find that the necessary first step is an adjustment for bias and quantification of uncertainty in dose estimates. So far, this is where most of our effort has been concentrated (2, 3). We describe our results in radiation dose estimation and comment on how the bias-corrected dose estimates that include quantification of uncertainty can be used in risk estimation.

Among occupational studies based on historical data, occupational radiation risk estimation is relatively "data rich". However, the data were collected for compliance rather than for estimation for individual doses. Consequently the bias can be substantial. Studies have shown that there was a systematic underestimation of doses for ORNL workers from 1945 to 1955 (2, 3). The first study (2) concentrated on dose estimation from film-badge data, and the second study (3) provided a systematic way of combining pocket-meter data with film-badge data for a better dose estimate. The results show that both bias and uncertainty vary widely between individuals and are poorly correlated with recorded annual dose. This suggests that the additional information contained in daily and weekly dosimetry records is needed for effective bias adjustment and quantification of uncertainty.

The dose estimate proposed for each individual is a probability distribution. This is the most general description of uncertainty and can be reduced to other descriptions of uncertainty. A nonparametric probability

distribution estimate, consisting of many (say 100) density points, can be reduced to a more concise description such as the five points of a boxplot, or to a few parameters of an assumed parametric distribution (such as a normal or a lognormal distribution). Each reduction is a loss of information and a gain in simplicity. These can be computed for an individual or for any cohort of individuals. Such generality allows the dose estimates to be useful for many purposes, including adjustment for dose uncertainty in epidemiological dose-response analyses by methods yet to be developed.

Our methodology is based on Bayesian estimation of "true dose" from available dose measurements in the form of a probability distribution. Bayesian dose distributions from individual measurements are combined with convolution computations to obtain dose distribution estimates for longer periods.

The Bayesian statistical approach estimates the unobserved quantities (true doses) given the values of the observed ones (recorded doses). A relationship between the true dose and the recorded dose in the form of a conditional probability distribution is the key element of the method. We begin by defining $P(x)$ to be the true dose distribution that concerns one individual in one measurement period. The key component for implementing our approach is the conditional probability distribution $P(z|x)$. In effect, $P(z|x)$ is the answer to the question: "If the true dose is x , what is the probability that the recorded value is z ?" This is determined by careful consideration of the properties of the measuring device (in this case the film badge or the pocket meter and the system used in reading and recording its dose). A necessary component is some information on the calibration error of the measuring device as well as recording practice. For the ORNL data, we assume a lognormal calibration error whose parameters are estimated from historical information. The rounding and censoring practices as well as use practices known from historical ORNL documents are included in the model of $P(z|x)$.

Note that $P(z|x)$ is a function of two variables, namely x and z , and it is constructed by specifying a distribution on z for each possible (fixed) value of x . After specifying $P(z|x)$ for all possible z and x , it is used as a function of x for each observed z . This is the "likelihood" of x for the observed z and is denoted by $L(x|z)$. Bayes's theorem then combines the likelihood $L(x|z)$ with prior distribution $P(x)$ to get $P(x|z)$, the posterior distribution of the true dose x given the recorded measurement z .

After the posterior dose distributions are obtained for each measurement period (in our case, a day or a week), the distributions must be "added" to compute a cumulative dose for a longer period. We compute yearly cumulative dose distributions, but other periods (such as a quarter to correspond to measurement periods in later years) may be used. Conditional on the recorded doses, the posterior distributions are independent and their convolutions can be computed efficiently with the discrete Fourier transform.

Because posterior distributions for individual measurements are often not symmetrical about the recorded measurement (in the ORNL case, especially the zero recorded doses), the cumulative distribution uncovers the bias in the added recorded doses.

When dose distributions for individuals are available, how can they be used in dose risk estimation? If we only wish to correct for bias in recorded doses, we can use the medians of the dose distributions and proceed with traditional dose-response analyses. Including dose uncertainty is more difficult. A Monte Carlo solution is possible but is also very computationally intensive. Other approaches that extend the dose estimation by Bayesian methodology into risk estimation can probably be developed. For example, some simple parametric assumptions about the dose distributions may carry through some known dose-response estimation methods. These may also require substantial computation, but less than the Monte Carlo solution.

A Monte Carlo simulation is more complex than it appears at first. The collection of risk estimates computed from samples generated by the individual dose distributions only accounts for the uncertainty in the data. We also need to account for error in the dose-response model. In traditional dose estimation, the model error is usually expressed by a confi-

dence interval. The Monte Carlo simulation must also account for the variability expressed by these intervals.

The Bayesian methodology developed in refs. (2, 3) can be used to quantify bias and measurement error in any situation, where it is possible to build a data generation model. In particular, the application of this methodology to dosimetry data at other facilities and for other periods may require relatively small modifications to the data generation models. The distribution convolution methodology remains the same.

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Advances in Environmental Dose Reconstruction

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Environmental dose reconstruction relies heavily on mathematical models to extrapolate information beyond the realm of direct observation. Because this extrapolation is inexact, quantitative uncertainty analyses are mandated, a feature that distinguishes environmental dose reconstruction from other kinds of exposure assessments. Table 1, for example, shows results in terms of 95% subjective confidence intervals for the

estimate of the thyroid dose for historic releases of ^{131}I from the Nevada Test Site, Hanford and the X-10 facility near Oak Ridge, TN.

The most advanced dose reconstruction projects are iterative; they use health risk as an assessment end point, and preliminary estimates of dose and risk are provided at an early stage. Table 2 shows preliminary results from Apostoaeci *et al.* (1) in which both thyroid dose and the excess lifetime risk of thyroid cancer are estimated for females born in 1952 who were exposed to both ^{131}I released from X-10 from 1952 to 1956 and ^{131}I deposited with Nevada Test Site fallout from 1952 to 1957.

Efforts are continually focused to improve the state of knowledge of those assumptions that affect the overall uncertainty in the final result. Informal or formal elicitation of expert judgment is often used to subjectively quantify uncertainty when direct measurements are not available. For example, a subjective probability distribution is required to describe the state of knowledge for the reduced biological effectiveness of ^{131}I with respect to an acute exposure to X rays or γ rays (1).

Effective project iteration requires the establishment of decision criteria to separate exposures of high and low priority for further investigation. At Oak Ridge, a decision criterion of an excess lifetime health risk of one chance in 10,000 (10^{-4}) has effectively directed screening calculations and permitted reallocation of limited resources among tasks. The iterative approach to environmental dose reconstruction ends with estimates of exposure, dose and health risk for which uncertainty is either acceptable or irreducible.

The state of the art in environmental dosimetry is reached when uncertain dose estimates are matched with the requirements for epidemiological investigations of the dose response (5). This entails the separation of uncertainty due to interindividual variability in dose among those individuals with similar exposure-identifying attributes, from lack of knowledge about the true but unknown quantities affecting the true dose for the individual or the true mean, variance and distribution of individual doses in the cohort.

TABLE 1
Comparison of the Results of Uncertainty Analyses among Five Different Dose Reconstruction Studies Involving Releases of ^{131}I

Location	Release (PBq)	Reference	95% subjective confidence interval		
			Lower bound (cGy)	Upper bound (cGy)	UF ^a (unitless)
Nevada test site (3,545 Utah school children)	5,600	3			
Maximum person			22	23 Gy	10
Median individual			0.33	19 cGy	7.6
Nationwide study (3,100 counties)		3			
Meagher Co., MT ^b	3.8		7.92 Gy	14.4	
Anderson Co., TN ^c	1.8		23 cGy	3.6	
Washington, DC ^c	1.2		21 cGy	4.2	
Hanford	27	(4)			
Maximum child			49	11 Gy	4.7
Typical child			1.9	52 cGy	5.2
HTDS (maximum of 841)		Kopecky ^d	33	4.77 Gy	3.9
HTDS (median of 841)			2.7	20 cGy	3.7
Oak Ridge dose reconstruction	0.96–1.1	1			
Solway ^e (13.5 km)			9.7	8.6 Gy	9.4
Buttermilk ^f (5.6 km)			21	4.73 Gy	4.5
Claxton ^g (22.7 km)			0.58	2.5 Gy	6.6
Regionally mixed commercial milk (all locations)			0.55	2.3 Gy	6.5

^a UF = uncertainty factor, which is the ratio of the upper bound of the 95% subjective confidence interval and the 50th percentile.

^b Female born in 1952 who consumed milk from a backyard cow.

^c Female born in 1952 who consumed milk from commercial dairies.

^d Personal communication.

^e Female born in 1944 who consumed milk from a backyard goat 13.5 km from X-10 in the Solway community.

^f Female born in 1944 who consumed milk from a backyard cow located 5.6 km from X-10 in the Buttermilk community.

^g Female born in 1944 who consumed locally produced commercial milk at 22.7 km from X-10 in the Claxton community.

TABLE 2
Preliminary Estimates of Thyroid Doses and Risks from Combined Exposure to ¹³¹I from NTS Fallout and X-10 Releases (Routine and Accidental) for Females Born in 1952 and on a Diet of Milk from Commercial Sources (1)

Location	Dose (cGy)			Excess lifetime risk		
	95% subjective confidence interval			95% subjective confidence interval		
	Lower level	Central value	Upper bound	Lower bound	Central value	Upper bound
Norwood (Anderson Co.)						
NTS (1952-1957)	1.8	6.5	22	2.7×10^{-4}	1.8×10^{-3}	1.4×10^{-2}
X-10 (1952-1956)	0.3	2.8	26	5.9×10^{-5}	7.3×10^{-4}	1.2×10^{-2}
Combined exposure ^a	3.2	11	35	4.4×10^{-4}	2.9×10^{-3}	2.3×10^{-2}
Gallaher Rd. (Roane Co.)						
NTS (1952-1957)	2.0	6.3	20	2.4×10^{-4}	1.8×10^{-3}	1.0×10^{-2}
X-10 (1952-1956)	0.6	4.9	40	1.1×10^{-4}	1.5×10^{-3}	1.5×10^{-2}
Combined exposure	4.4	13	50	5.9×10^{-4}	3.7×10^{-3}	2.2×10^{-2}
Lenoir City (Loudon Co.)						
NTS (1952-1957)	1.7	5.4	17	2.8×10^{-4}	1.5×10^{-3}	7.9×10^{-3}
X-10 (1952-1956)	0.3	2.0	14	5.1×10^{-5}	5.2×10^{-4}	6.3×10^{-3}
Combined exposure	3.2	8.4	23	4.6×10^{-4}	2.4×10^{-3}	1.3×10^{-2}
Solway (Knox Co.)						
NTS (1952-1957)	2.0	5.0	12	2.6×10^{-4}	1.4×10^{-3}	6.5×10^{-3}
X-10 (1952-1956)	0.7	5.1	34	1.2×10^{-4}	1.4×10^{-3}	1.2×10^{-2}
Combined exposure	4.0	11	40	5.1×10^{-4}	3.3×10^{-3}	1.9×10^{-2}

^a The combined exposure is obtained through summation over the full distributions of values; therefore, the results will differ from simple totals of the values in the columns.

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Dosimetry at Low Dose Rates: Biological and Molecular Approaches

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Most environmental exposures in the nuclear industry, during environmental cleanup, and during space flight are delivered at low dose rates either from external sources or from internally deposited radioactive materials. It is important to recognize that all biomarkers are not useful for evaluating such past exposures. Biomarkers can be subdivided depending

on their applications. The classic breakdown of biomarkers is: markers of exposure and dose, markers of risk or susceptibility, and markers of disease. There is often confusion when determining which detected biological changes are applicable to each category. Markers of exposure and dose can be used to reconstruct unknown exposures and predict past exposure when no physical measurements of dose were available at the time of an accident. Markers of risk or susceptibility can identify sensitive individuals in a population who are at increased risk for a given insult. Finally, markers of disease represent the initial cellular or molecular changes that occur during the early stages of a disease. These can often be detected prior to clinical expression of the disease and are useful in disease detection and treatment. There is some crossover between categories, but each end point seems to have a primary biomarker function. This presentation will be limited to markers used to estimate dose and exposure. However, in some cases the biological change also provides an indication of increased risk and individual susceptibility.

It is critical to develop markers that are useful for detection of low-dose-rate exposures. For biological damage to be useful as a dosimeter, it must provide an integration of the total exposure with time. Thus many transient changes detected after a single acute radiation exposure are not useful markers of low-dose-rate exposures. A large number of approaches have been evaluated as potential biomarkers of exposure. These include changes observed at the cellular and chromosome level, mutation frequency, and more recently a range of molecular and chemical methods. Examples of each of these are discussed here.

One of the oldest and still one of the most promising techniques is the use of chromosome damage to detect past radiation exposure. The major requirements for this technique are the availability of dividing cells and the accumulation of metaphase cells that can be prepared so that each chromosome can be evaluated for potential breakage or translocations. This technique has been used widely for dose reconstruction (1) and has undergone major changes and development that make it very useful for evaluation of low-level exposures of populations. It was determined that there are stable and unstable aberrations and that in dividing cells unstable aberrations increased as a function of dose rate while stable aberrations accumulated as a function of total dose. With the advent of fluorescence

in situ hybridization (FISH) techniques, it became possible to label whole chromosomes and to score stable chromosome translocations with much greater accuracy. As the techniques have continued to develop, every human chromosome can be painted (multi-FISH), and equipment is being developed to aid in scoring these aberrations (2).

An additional cytogenetic technique developed to evaluate chromosome damage is the induction of micronuclei. This technique is very rapid and detects mostly chromosome deletions. It has been automated using flow cytometry techniques for polychromatic erythrocytes and lymphocytes, and research is expanding to use it for additional tissue types. Such a technique has great potential for rapid evaluation of large populations after an accidental or wartime exposure but is more limited in its application to low-dose-rate exposures. It is also possible to use micronuclei to relate different exposures, α -particle traversals, energy deposition patterns, and dosimetric measurements and to evaluate the distribution of energy in a system like the respiratory tract (3).

A technique currently being developed for the evaluation of cytogenetic damage is called comparative genomic hybridization (CGH). In this technique DNA from the damaged or diseased tissue is extracted from nondividing cells and hybridized against the chromosomes of a normal metaphase cell. Areas of the chromosome that are lost, translocated or duplicated can be identified. Current data suggest that the technique has limited usefulness as an indicator of exposure. Its major application is for the detection of cytogenetic changes associated with the development of cancer and loss and duplication of specific chromosome regions associated with the disease.

There have been a number of techniques developed based on the induction of gene mutations. Among the mutations most frequently assessed are those at the *HPRT* and the glycophorin A loci (4). These have been applied to large human populations and seem to be useful for identifying exposed or nonexposed populations. However, large differences between individuals make it impossible to use these techniques for individual dosimetry.

The field of molecular toxicology has been expanding rapidly and has resulted in methods to better characterize cellular and tissue responses to a wide range of toxicants that produce free radicals, including ionizing radiation. There are a number of stress-response genes that are induced by free radicals and that are thought to be linked to the induction of cancer. Expression of some of these genes remains elevated for long periods after they are activated by stress. To measure changes in gene expression, new techniques have been developed where expression sequence tags (ESTs) are bound to "chips" for detection of changes in expression in large numbers of genes (5). Such techniques simultaneously evaluate the expression of a wide array of genes that are important in toxicant metabolism, free radical handling, cellular proliferation and DNA repair. There is little information available at present on the exposure-response relationships associated with these changes, but with additional development the technique may have wide application.

There is a wide range of molecular and chemical techniques that are being developed to measure changes in DNA structure and individual bases which may have use in biological dosimetry. Immunochemical recognition of radiation-induced thymine glycol using capillary electrophoresis and laser-induced fluorescence detection has been shown to be very sensitive. It has a detection limit of 3×10^{-23} mol, which suggests that the technique can detect one thymine glycol per 10^9 bases (6). Fourier-transformed infrared (FT-IR) spectra have proven useful in evaluation of the total amount of structural DNA damage in a tissue. This approach has been applied successfully to a wide range of tumor types. The infrared technology has been shown to be a powerful means of discriminating between normal prostate tissue, benign hyperplasia and prostate cancer (7). There are limited data on exposure-response relationships for the induction of DNA abnormalities, but the technique has promise since it can be applied to small samples, is very sensitive, and does not require live or dividing cells. However, there are serious questions as to how long the DNA alterations remain in the cell population, which may limit the usefulness of the technique for biodosimetry.

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DISCUSSION: Dosimetry at Low Dose Rates

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The papers presented are excellent reviews for three of the significant specialties within radiation dosimetry. They fall into two broad categories:

1. Occupational and biodosimetry
 - a. Begins with data collected at (or within) the receptor
 - b. Projects dose to the target organs for individuals
2. Environmental dosimetry
 - a. Begins with data collected at the emission source
 - b. Projects dose to receptor's target organs by way of environmental pathway analysis

Dr. George Ostrochov's paper on measurement error in occupational dosimetry makes clear the need in dose-response analysis to adjust for bias and uncertainty. His domain is "data rich"; however, all the data have usually been collected for compliance, not for the epidemiological purpose for which he and his colleagues are now using the data. His Bayesian approach seeks to determine the probability distribution for the "true dose" for each worker, given the recorded film-badge and pocket-chamber data. Dealing with voluminous archived data is a great challenge. He points out that, in the decade 1945-1955 alone, he has 30,000 person-years of hard-copy data for the Oak Ridge National Laboratory worker study. And then comes the really "tough" part: estimating the risk distribution from the cumulative dose distribution for each worker, an area where new statistical methodologies certainly need to be developed.

The dosimetry associated with environmental dose reconstruction that Dr. Hoffman reviewed demonstrated heavy reliance on mathematical modeling to extrapolate beyond radionuclide releases at the source, and

on limited environmental monitoring data. The goal in this field is two-fold: to determine the risk for real people or "representative" individuals and to provide individual dose for epidemiological studies. A fundamental problem he must confront is that of treating the very large uncertainties in the individual dose estimates. Here we mean the uncertainty in the dose due to our lack of knowledge of numerical estimates on the model parameters, as opposed to the "true" interindividual variability of dose; uncertainty and variability are often confused. An iterative approach is taken, as Dr. Hoffman indicates, in current state-of-the-art environmental dose reconstructions for determining dose and risk distributions for representative individuals, that is hypothetical people who match a variety of scenarios (diet, lifestyle, age, location, etc.). The approach is iterated until the uncertainty becomes acceptable, or the resources are exhausted!

Looking at both of the above presentations, one must ask whether a paradigm shift is taking place. Are we moving in the direction of making decisions on risk management, epidemiological power analysis or the like, based on distributions of dose or risk, rather than point estimates, i.e. some ill-defined "best estimate"? I will only pose that question rhetorically.

Dr. Brooks has provided us with an excellent summary of the many techniques currently used in biodosimetry. He made the point that molecular markers of exposure or dose must provide integration of dose with time; those which arise from transient responses are not helpful and may indeed provide misleading data. The most promising biomarkers of exposure are the stable aberrations that arise from cumulative chromosome damage after irradiation. The most popular of these techniques include induction of gene mutations, such as *HPRT* and glycoporphin A assays. Currently, they are most useful in separating exposed from control groups rather than for individual dosimetry. A biomarker that is new to most of us who do not follow this field closely is that of early-response genes that are induced by production of free radicals within the cell and are therefore useful for assessing chemical and radiation-induced damage. This marker quantifies the array of changes in gene expression.

Some of the new techniques under development include PCR and "Pac-Man" techniques. In principle, these are ultrasensitive biomarkers of exposure, but they still need further development before becoming practical in the field. One technique that Dr. Brooks did not mention is that of electron spin resonance (ESR), sometimes referred to as electron paramagnetic resonance (EPR). We will be hearing more about this technique since it is so useful and is being applied in some of the Russian studies as well as in the studies of the Japanese A-bomb survivors. The basic principle is straightforward; free radicals are produced in the hydroxyapatite crystals which make up bone and teeth. The point defects produced in these crystals by radiation yield long-lived free radicals, characterized by unpaired electron spins which give rise to the signal in an ESR spectrometer. The strength of the signal is proportional to the radiation absorbed dose, and the free radicals are stable at physiological temperatures for times longer than the human life span. Although I am not a researcher in this area, I would suppose that in principle the neutron dose could be discriminated from the γ -ray dose, since the mechanism for radiation-induced damage would be distinctly different in the two cases, and I would expect a resonance signature that would differentiate the two types of radiation.

In summary, there is at least one pressing issue in each of the specialties reviewed here. I would like to encourage work in the near future on the following:

Occupational: Dosimetry that integrates internal with external dosimetry. The organ's target cells certainly do not distinguish radiation arising from within from that arising from outside the body. Although traditionally those concerned with occupational dosimetry and epidemiology have dealt with external dosimetry because that is what is registered on film badges and pocket chambers, in many cases, the radiation dose arising from internal exposure which does not register on the personal dosimeter, e.g. α particles, may be a significant contributor to the overall radiation dose.

Environmental: The uncertainties are exceptionally large. Those who work in this area are to be commended for confronting the uncertainties

in dose and risk head-on, despite all the difficulties. Dr. Hoffman has certainly been a leader in this effort. It was much easier in the "good ol' days" when only a single number was provided for a person's dose. But now that we have doses and risks with their large uncertainties, how do we best incorporate them into our risk assessment and management planning and analyses? What can we do to reduce these uncertainties, which in some cases are so large as to leave doubt as to whether we answered the question "What is the dose or risk?" in the first place.

Biodosimetry: Although much has been achieved in this field, much more is still under development—with a promise to provide reliable dosimetry for occupational and environmental epidemiology "soon". I believe that biodosimetry is poised to provide a lot of help to these epidemiological and risk assessment arenas now and in the near future. Indeed, I would argue that it must. Both environmental and occupational dosimetry, especially the former, are in urgent need of having biological checks of their validity. Perhaps the most important role for biodosimetry is that of challenging the results of the rigorous modeling that is currently used to produce doses and risks for occupational and environmental studies. Dr. Dale Preston from the RERF has spoken of the important role played by conventional chromosome aberration analysis in discovering significant discrepancies in the dosimetry, as modeled by the DS86 algorithm.

V. NEW PARADIGMS FOR LOW-DOSE RADIATION RESPONSE?

Chair: Jerome S. Puskin, *Environmental Protection Agency*

The Use of Chromosome Rearrangements to Evaluate Genomic Instability

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(with J. J. Corcoran, M. I. Kaplan, A. Hartmann and C. L. Limoli, *Lawrence Berkeley National Laboratory*)

It is widely accepted that carcinogenesis is a multistep process consisting of initiation, promotion and progression, and that malignancies arise as a consequence of an acquired genetic change (genomic instability) in a cell that is capable of clonal expansion. Genomic instability, for the purpose of this presentation, is defined as the increased rate of acquisition of alterations in the mammalian genome. Exposure to ionizing radiation can increase cancer incidence, and in a number of *in vivo* and *in vitro* model assay systems, exposure to ionizing radiation also leads to genomic instability. Instability manifests in clonal isolates of surviving cells as a persistent reduction in plating efficiency (lethal mutations or delayed reproductive cell death), increased giant cell formation, increased micronuclei, gene amplification, transformation, mutation rate, and/or changes in chromosome number or structure (reviewed in ref. 1). The molecular, genetic and cellular events induced by ionizing radiation that lead to destabilization of the genome are not known. A number of events occurring within a cell as a consequence of irradiation may initiate genomic instability, e.g. DNA damage, signal transduction cascades, gene induction or deletion, or even activation of a latent virus endogenous to the irradiated cell. Genomic instability, as measured by a number of different end points, is a relatively frequent event in the progeny of cells surviving irradiation, which suggests a large target size or multiple targets for its induction.

We have been investigating the mechanisms and biological consequences of X-ray-induced chromosomal instability in the progeny of cells surviving irradiation. Our model assay system uses human-hamster hybrid GM10115 cells which contain a single copy of human chromosome 4 in a background of 22-24 hamster chromosomes. After irradiation a clone is said to be unstable when three or more subpopulations of meta-

phase cells are observed that show unique rearrangements of the human chromosome and comprise $\geq 5\%$ of the total number of metaphase cells analyzed. Because these unique subpopulations of metaphase cells arise during clonal expansion of the exposed cell, they are considered a delayed effect of the initial exposure to the noxious agent.

There is ample evidence that DNA double-strand breaks are the primary lethal lesion induced by ionizing radiation and also lead to chromosomal rearrangements. Consequently, we have investigated the role of DNA strand breakage in the induction of delayed chromosomal instability. Using cells of a GM10115 cell line containing a single copy of human chromosome 4 that was hemizygous at the *APRT* locus, Limoli *et al.* (2) electroporated restriction enzymes into cells and selected for mutations at the *APRT* locus. An enzyme-induced mutation indicated that the restriction enzyme used had entered the cell and cleaved the DNA, and the break had been processed by the cell to produce a mutation. Implicit in this is that the restriction enzyme induced a number of other breaks in the genome, and that these breaks were not lethal to the cell. These clonal isolates were then analyzed cytogenetically for any changes in human chromosome 4 occurring during clonal expansion of the mutant. However, analysis of 150 independent clones surviving exposure to four different enzymes revealed no chromosomal instability. In contrast, when cells were treated with other agents known to produce DSBs, e.g. X rays, the radiomimetic drug bleomycin or neocarzinostatin, or photochemical processes, chromosomal instability was observed in a high frequency of surviving clones. DSBs are rapidly repaired in mammalian cells (3), so it is unlikely that damage induced by these treatments can persist over multiple cell generations and initiate new chromosomal rearrangements in subsequent cell generations. Whatever the molecular or cellular event initiating this process, we have evidence to suggest that in our GM10115 cells it is nuclear in origin. DNA lesions, possibly complex DSBs or DSBs occurring in the presence of other DNA interactions induced by X rays or radiomimetic agents, can ultimately induce the unstable phenotype. Clearly, the appropriate circumstances necessary for induction of instability are not induced by restriction enzymes.

Once a radiation-induced initiating event has occurred in a surviving cell, instability can then be perpetuated in cells by chromosomal recombination involving interstitial repeat sequences, followed by bridge-breakage and refusion cycles, which serves to maintain the observed instability over multiple generations (4). Because cancer cells can exhibit multiple end points associated with genomic instability, we have also investigated the potential relationship between chromosomal destabilization and the other end points of genomic instability. We generated a series of chromosomally stable and unstable GM10115 clones by exposure to X rays. These clones were then subjected to a series of assays to determine if chromosomal instability is associated with a general "mutator phenotype" and if it modulates other end points of genomic instability. Clones were analyzed for sister chromatid exchange frequency, delayed reproductive cell death, delayed mutation, mismatch repair and delayed gene amplification. Statistical analyses performed on each group of chromosomally stable and unstable clones indicated that, although individual clones within each group were significantly different from unirradiated clones for many of the end points, there was no significant correlation between chromosomal instability and sister chromatid exchange, delayed mutation and mismatch repair. Delayed gene amplification was found to be marginally correlated to chromosomal instability ($P < 0.1$), and delayed reproductive cell death (the persistent reduction in plating efficiency after irradiation) was found to be significantly correlated ($P < 0.05$) (5). These correlations may be explained by chromosomal destabilization, which can mediate gene amplification and can result in cell lethality. These data implicate multiple molecular and genetic pathways leading to different manifestations of genomic instability in cells surviving exposure to ionizing radiation.

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The Relationship between Radiation-Induced Instability and Radiation Carcinogenesis

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We have recently proposed a model that identifies radiation-induced genetic instability as the earliest cellular event in the multistep sequence leading to radiation-induced cancer (1). This initial instability puts all genes at risk for mutation, but its major impact on carcinogenesis occurs when critical genes, such as *Trp53* (formerly known as *p53*), are mutated as a secondary consequence of the radiation exposure. This model is linked to the growing body of studies describing the delayed appearance of mutations and chromosomal abnormalities in the progeny of irradiated cells (2-4). In these studies the latent expression of chromosomal aberrations and mutations in these progeny has been interpreted to be a manifestation of genomic instability induced by radiation. The present paper reviews data from this laboratory that initially led to the proposal of this model, and subsequent studies aimed at testing predictions of this model.

Studies were initially designed to test whether alteration of the *Trp53* gene was a significant early event in radiation-induced mammary cancer in mice. The focus of these studies was on EF42, a clonally derived radiation-induced growth variant isolated from mouse mammary tissue after whole-body irradiation. Having established the preneoplastic nature and neoplastic potential of EF42 cells, we conducted a series of studies designed to investigate their preneoplastic to neoplastic transition in relation to mutations in *Trp53* (1).

To characterize the *Trp53* mutations in EF42 cells, we first identified mutations in clonal isolates of EF42 cells in which virtually all cells appeared to contain mutant protein (based on immunohistochemistry). Clonal isolates were used because they were considerably less pleomorphic than the EF42 cells. Three mutations were identified using direct sequencing of PCR products: a transversion at codon 173 from TGC to TGG leading to a substitution of tryptophan for cysteine (C173W); a transition at codon 278 from GAC to GGC, resulting in a substitution of glycine for aspartic acid (D278G); and a transversion from GTT to GAT at codon 269 resulting in a substitution of aspartic acid for valine (V269D). Since each of these mutations created new restriction sites, we next analyzed EF42 cells using PCR-RFLP analysis as a function of passage and neoplastic potential.

In these studies, we found that multiple mutations in *Trp53* occur before the acquisition of the neoplastic phenotype. At passage 11, both the C173W and D278G were present at a frequency of 8%. Interestingly, V269D was detected only in the clonal isolates derived from EF42 cells.

The growth advantage conferred to cells with the D278G mutation was striking. The selective expansion of these mutant cells was also accompanied by loss of heterozygosity (LOH) at the *Trp53* locus and amplification and overexpression of *Myc* and cyclin D1 as well as numerous chromosomal alterations associated with cytogenetic instability.

Although it was clear from the data that *Trp53* mutations represent critical early events in the neoplastic progression of EF42 cells, our data argued that these mutations were not induced directly by radiation but arose in the progeny of irradiated cells several generations later. Because of the clonal origin of EF42 and the clear growth advantage conferred by *Trp53* mutations in these cells (particularly D278G), *Trp53* mutants should have comprised virtually 100% of the cells at the earliest passages examined if it had been induced directly. Rather, *Trp53* mutants comprised only 8% of the cell population at passage 11 and were undetectable at passage 6 using PCR-RFLP or the more sensitive probe shift assay. These results led us to propose our model, which identifies genomic instability as the earliest cellular event in the multistep sequence leading to radiation-induced cancer. This initial instability puts the structural integrity of virtually all genes at risk for mutation, but its major impact on carcinogenesis occurs when critical genes, such as *Trp53*, are mutated as a secondary consequence of the radiation exposure. Such mutations lead to further instability. To our knowledge, these data represented the first evidence for delayed mutations in a gene directly involved in the cancer process.

As a first approach to examine the role of radiation-induced genomic instability in development of mammary cancer, we undertook a series of studies aimed at determining whether instability could, in fact, be induced in mammary epithelial cells and to obtain information on the time course of its manifestation after radiation exposure (5). For these studies, primary mammary epithelial cells were isolated using techniques common to our laboratory for the analysis of altered growth phenotypes. Subsequent to isolation, cells were irradiated in suspension with 3 Gy, plated and analyzed. Rather than studying individual colonies cytogenetically (which would not have been possible with these primary cells) and determining nonclonal cytogenetic alterations, it was necessary to grow the cells in mass culture and examine the population at various times for the appearance of chromatid aberrations. The frequency of chromatid aberrations remained relatively constant in nonirradiated control cells over the entire period. The frequency of chromosome aberrations (principally dicentric) was, as expected, high at 4 population doublings but had decreased substantially by 8 doublings to control levels, demonstrating the recovery from initial radiation damage. However, an increase in chromosome and chromatid aberrations was observed at about 20 doublings which persisted through 28 doublings. These data clearly demonstrated the induction of delayed cytogenetic instability.

Having previously established that genetic differences in their sensitivities to radiation-induced mammary cancer are based on inherent differences in sensitivities to transformation, we next undertook studies to examine radiation-induced cytogenetic instability in these two strains of mice (5). It was reasoned that if genomic instability was, in fact, linked mechanistically to radiation-induced mammary cancer, then target cells from cancer-susceptible mice would exhibit a greater susceptibility to radiation-induced cytogenetic instability than cells from mice resistant to induction of mammary cancer. To test this prediction we examined the appearance of delayed cytogenetic instability in γ -irradiated mammary epithelial cells from C57BL/6 mice and compared these results with effects in cells from BALB/c mice. Results for the cells from BALB/c mice were similar to those already described. The yields of dicentric and total aberration frequencies early after irradiation in the irradiated cells from C57BL/6 mice were similar to those observed for those from BALB/c mice and, as in BALB/c mice, between 3 and 9 population doublings, these frequencies were reduced to control levels. Significantly, unlike the response of cells from BALB/c mice, the frequency of chromatid aberrations in both control and irradiated cells from C57BL/6 mice was similar over the entire experiment (28 population doublings). To our knowledge these data were the first to correlate a specific form of genomic instability with strain-dependent differences in susceptibility to radiation-

induced neoplastic transformation. Such results further strengthen the hypothesis that genomic instability is linked to the mechanism of radiation-induced cancer as a critical early event.

More recently we have completed a series of studies examining the induction of cytogenetic instability in F₁ hybrids of BALB/c and C57BL/6 mice. Such studies were conducted to determine whether susceptibility to induction of cytogenetic instability was, in fact, a heritable trait and to provide initial data on the pattern of inheritance. The F₁ hybrids were similar to the C57BL/6 mice in that both hybrids were resistant to induction of instability. These data provide further support for our model, and for our proposed use of genetic analysis to examine the gene(s) involved in the strain-dependent differences.

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Genomic Instability, Cell Death and Mutagenesis

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Early studies on the cytotoxicity of ionizing radiation showed that reproductive cell death occurred as many as five or six generations after exposure (1-3). Some cells capable of forming viable colonies displayed a consistently reduced capacity to produce viable colonies when subcultured (4, 5). This phenomenon, now known as delayed expression of lethal mutations, has been observed in mouse, hamster and sheep cells, and in human \times HeLa hybrid cells among others (6, 7). The phenotype of delayed lethal mutation persists in individual clones for many generations after the initial exposure, and has been detected by serial subcultivation of clonal survivors. In Chinese hamster ovary (CHO) cells, delayed lethal mutation was a dominant phenotype (8).

Quantitative mutation assays have traditionally determined the number of clonogenic cells bearing a specific-locus mutation in a population of cells seeded and screened at an early, fixed time after mutagen exposure. An extensive series of studies have been done to quantify radiation-induced mutations at early times after exposures, and careful molecular analysis of these mutations suggests that while ionizing radiation can cause point mutations of all types, the majority of radiation-induced mutations exhibit large-scale loss of genetic material, through either deletion or recombinational mechanisms. In many studies of radiation-induced mutations at early times after exposure, the typical mutant clone appeared to be homogeneous in its genotype.

Using a modified experimental protocol, specific-locus mutations have been shown to arise at elevated rates or unusually high frequencies in CHO cells and in human lymphoblastoid cells for many generations after exposure to ionizing radiation (9-12). It has been suggested that these delayed mutations may arise via the induction of genomic instability.

Several lines of evidence support the hypothesis that ionizing radiation induces a persistent genomic instability. This instability is often described in terms of subclonal karyotypic heterogeneity (7; also see abstract by Morgan on p. 111). To unambiguously identify X-ray-induced

genomic instability in human TK6 lymphoblasts, Luria-Delbruck fluctuation analysis was used to measure mutation rates at the *TK* locus in clones exhibiting karyotypic heterogeneity and complex aberrations (11). The mutation rates in the cells with karyotypic instability were compared with the spontaneous mutation rate in the unirradiated TK6 cells. Unstable clones with complex aberrations were shown to have elevated mutation rates. Molecular analysis of the *TK* mutations showed an elevation in both intragenic mutations and chromosomal-scale mutations with loss of heterozygosity inclusive of the *TK* locus. Another study of the nature of delayed mutations arising at the *Hprt* locus in CHO cells demonstrated that these mutations did not resemble typical mutations arising at short times after exposure to ionizing radiation, as the majority of the delayed mutants showed small changes that more typically reflect an elevation in the spontaneous mutation rate (12).

Taken together, these studies suggest that radiation exposure can produce a persistent instability in the genome that is associated with the induction of a mutator phenotype. This mutator phenotype permits the ongoing emergence of karyotypic variants and specific-locus mutations at least 60–100 generations after the irradiation. These studies demonstrating the ongoing cellular “memory” of the initial radiation exposure may help explain the apparent discordance between the relatively low frequencies of specific-locus mutations at early times after exposure, the high frequencies of initiated thyroid and mammary epithelial cells that others have reported (13, 14), and the emergence of clonally derived tumors that are known to require the accumulation of a series of mutations within a single cell.

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Bystander Effects of Radiation: Mechanisms of Action and Significance in Risk Assessment

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Current uncertainty in assessing the risk of exposure to ionizing radiations is due largely to a lack of knowledge of mechanisms and an understanding of human variability, attributable in part to genetic heterogeneity among individuals. This is especially true for low-dose/low-dose-rate exposures to ionizing radiation. Radiation biology and related risk assessment have progressed through a series of paradigms. The first clearly identifiable radiation paradigm was target theory. Originally designed to explain the shape of cell survival curves, target theory postulated that within cells are sensitive regions critical to a cell's survival. A cell dies when these sensitive regions are damaged by radiation. Each sensitive region was called a “target”, while an energy deposition event capable of inactivating a target was defined as a “hit”. Mathematical models derived from target theory produced excellent fits to cell survival curves, perhaps explaining the popularity and longevity of this paradigm. Framed by target theory, the scientific goals then were to understand the physical and chemical nature of “hits” and the biological nature of targets. While much progress was made, the paradigm ultimately failed for two reasons. First, convincing evidence was obtained demonstrating DNA to be a critical molecule for radiation effects, yet target theory could not easily incorporate DNA into the models. The final demise of target theory occurred when cellular recovery processes, for which target theory had made no allowance, were discovered. This set the stage for the second radiation paradigm. Unlike the nearly mechanical view of target theory, the repair paradigm acknowledged that radiation-damaged cells “fight” for survival. Research priorities then shifted toward understanding the biological nature of recovery, now nearly synonymous with DNA repair. Undoubtedly, the repair paradigm has stimulated much productive research. However, it too has come under serious challenge lately since DNA repair alone provides no convincing explanation for newly discovered cellular responses such as genomic instability. Further, the presupposition that a proportionality between high-level exposures to ionizing radiation (>0.1 Gy) and the health consequences of low-level ionizing radiation continues to dominate in most scientific and regulatory circles. According to this view: (1) Radiation is harmful at all doses, i.e. the linear no-threshold theory, and (2) there are no effects at low doses that cannot be predicted from effects observed at high doses. Unaccommodated by these contentions is the possibility that the detrimental effects of ionizing radiation may occur only above a threshold dose and/or that low-level ionizing radiation may induce responses that may be adaptively beneficial or otherwise nonpathogenic. Further, the risk paradigm does not account for the possibility that persistent untoward responses may be elicited by low-level ionizing radiation that are not observed with higher doses of ionizing radiation. Moreover, it does not take into account that at least some biological effects of ionizing radiation can be induced in cells that receive no direct exposure, and it ignores the potentially decisive role of genetic variability in responses to low- and high-dose ionizing radiation in terms of their consequences.

The current debate over radiation hormesis demonstrates well that even the most basic questions regarding the long-term health effects of low-dose exposures cannot be answered with confidence. Indeed, recent observations call into question the very foundation of some microdosimetric models. For example, the Human Respiratory Tract Model for Radiological Protection is built on an underlying assumption that traversals of α particles through the nuclei of airway target cells alone cause cancer. Yet biological effects have been seen in cells not directly exposed

to radiation (e.g. 1-3), a phenomenon variously called a "bystander" or "non-targeted" effect, suggesting that more cells may be at risk than previously thought. Recognition that active biological responses are initiated by ionizing radiation serves as a foundation of an emerging radiation paradigm. The new paradigm postulates that effects of low radiation doses may be determined by cellular processes that are activated by exposure. In this view, the initial low radiation dose may have little effect other than to trigger these processes, which can include both gene transcription and translation. As of now, however, the biochemical pathways stimulated by ionizing radiation remain poorly understood.

Recent investigations have revealed several potentially detrimental phenomena related to low-level ionizing radiation that would not necessarily be predicted from prior studies in which the effects of radiation were examined at higher doses and dose rates. As examples, a low dose (~1 cGy) of high-LET α particles like those emitted by radon and radon progeny can induce genetic damage in rodent and normal human lung cells, as indexed by increases in sister chromatid exchanges (SCEs), in the absence of direct nuclear or even whole-cell hits (4, 5). Such a low-dose effect is associated with the induction of elevated levels of both extracellular and intracellular reactive oxygen species (ROS), particularly superoxide anions and hydrogen peroxide (3). Of significance, both the SCE and ROS responses can occur as a bystander effect in unirradiated cells through the actions of superoxide dismutase (SOD)-inhibitible transmissible factors present in irradiated medium and in the supernatants of irradiated cells (1, 2). These findings and others (6) implicate an important role for ROS in mediating DNA damage in response to low-level ionizing radiation, and they demonstrate that detrimental effects can be induced indirectly in unirradiated cells, i.e. the bystander effect, to the same extent as found with irradiated cells (1-3). Newer evidence suggests that bystander effects in these model systems may be mediated via cytokines that are up-regulated downstream of the ROS response.

Evidence that soluble extracellular mediators can cause DNA damage comes from several observations. For example, conditioned medium from cells from patients with the cancer-prone disorders Bloom's syndrome, Fanconi's anemia and ataxia telangiectasia cause chromosomal aberrations and increased SCE (e.g. 7). Radiation-induced clastogenic activity was first observed 30 years ago by Hollowell and Littlefield (8) in patients receiving radiotherapy and by Goh and Sumner (9) after accidental total-body irradiation. Activity has also been observed in atomic bomb survivors and Chernobyl residents (10, 11). Clastogenic factors are present in the cell culture medium after irradiation that may induce chromosome breakage when the medium is transferred into recipient cell cultures. These clastogenic factors are remarkably persistent and are often still in evidence months, years or even decades after exposure (12). *In vitro* experiments have shown that irradiated plasma yields clastogenic activity as well. Experiments in which rats were exposed to whole-body irradiation (2.5-4 Gy) demonstrated clastogenic factors in plasma from at least 15 min to 10 weeks, the earliest and latest times (13); dilution of irradiated plasma with unirradiated plasma did not reduce the number of chromosome aberrations. Clastogenic factors are also produced in patients with chronic inflammatory disease and in viral infections like HIV. A variety of chemical species have been proposed to function as clastogenic factors, e.g. aldehydic breakdown products of lipid peroxidation, the cytokine tumor necrosis factor- α (TNF- α), and inosine nucleotides. Aside from TNF- α , clastogenic factors generally have a low molecular weight. They can induce damage in living cells but not in isolated DNA, which suggests that clastogenic factors modulate cellular functions that lead to their DNA-damaging effects. Like the radiation-induced transmissible factors we are studying, the effects of clastogenic factors can be inhibited by SOD (10), which provides a further link between ROS and DNA damage associated with clastogenic factors.

Other examples of responses consistent with bystander effects associated with ionizing radiation include the demonstration that increases in Tp53, which are related to DNA strand breaks, occur in higher percentages of cells than those experiencing a nuclear hit by an α particle (14), that supernatants transferred from irradiated cells to unirradiated cells can

diminish the survival of the latter (15), and that genomic instability can occur in more cells than expected based on target theory alone (e.g. 16).

The above studies were performed *in vitro*. Even so, some limited evidence suggests that transmissible factors may be operational in mediating bystander effects *in vivo* as well. Nagarkatti and coworkers (17), for example, have recently reported that exposure of mice to radon causes alterations in extra-pulmonary cell populations. Such findings are consistent with the possibility that exposure to α particles can result in the generation of biologically active, diffusible products that may act at unirradiated body sites. What remains to be demonstrated is that disease processes such as carcinogenesis can be a consequence of bystander effects in the body that are associated with ionizing radiation. Ultimately, the identification and characterization of the mechanisms that underlie bystander effects will be prerequisite to subsequently determining how genomic and "proteomic" variants in the human population may contribute to their potential untoward effects.

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DISCUSSION: New Paradigms for Low-Dose Radiation Response?

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In recent years several new paradigms of radiation action have been demonstrated which challenge some of the traditional tenets of radiobiology. Insofar as these tenets have been used as guides for the extrapolation from risks of cancer at high doses to those at low doses, such an extrapolation should be re-examined in terms of the new paradigms.

Among these paradigms (with selected references) are: (a) low-dose hypersensitivity (1); (b) radiation-induced reduced plating efficiency (delayed reproductive death) (2); (c) inverse dose-rate effect (3); (d) induction of genes by radiation (4); (e) bystander effect (5); and (f) radiation-induced genomic instability (5). Most of these topics have been covered in detail by others in this session.

The topic of low-dose hypersensitivity has not been discussed. This discovery challenges the traditional view of the shapes of survival curves for mammalian cells. Apart from survival curves that are exponential, the initial curvature of survival curves for mammalian cells indicated that cells were more resistant at low doses of radiation. The advent of new technologies enabled a closer examination of survival at low amounts of cell killing, and for many cell lines it was found that there was an initial portion of the survival curve in which hypersensitivity was evident (1). The authors interpreted this to indicate that low doses of radiation (<0.2 Gy) induce a protective mechanism.

An example of such a protection mechanism showing the effect at a molecular level in cellular DNA has been published recently (6). The authors developed an assay for *cis*-thymine glycol (Tg) that is sensitive down to zeptomolar levels. They were able to show that 4 h after a priming dose of radiation (0.25 Gy), human lung carcinoma cells (A549), after a dose of 1 Gy, removed Tg from their DNA more rapidly than without the priming dose.

The new paradigms challenge some of the basic tenets of radiobiology (paradigms lost): (a) Low-dose hypersensitivity goes against the notion that at lower doses cells are, if anything, more resistant than at higher doses. (b) Reduced plating efficiency after irradiation goes against the assumption that the plating efficiency of cells after irradiation is the same as that of the unirradiated cells—that assumption is inherent in the interpretation of split-dose experiments, measurements of TD_{50} , etc. (c) Inverse dose-rate effect for high-LET radiation is the reverse of that expected from the well-known low-dose-rate sparing for low-LET radiation. (d) Radiation hormesis has been framed in several forms; now there is evidence at the molecular level that repair of radiation-induced damage can be induced by low doses (however, it should be noted that the damage whose repair was shown to be accelerated by a priming dose is not recognized as being radiobiologically significant). (e) The bystander effect shows that cells within which no radiation energy is deposited react to energy deposition events in their vicinity—this effect also challenges radiobiological tradition. (f) Genomic instability appearing in some of the progeny of an irradiated clone several generations after irradiation challenges two generally held ideas—that all chromosome rearrangements are evident one or two cell divisions after irradiation, and that cells derived

from a clone have similar genetic makeup (all would have the same chromosomal stability).

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VI. KEYNOTE ADDRESS

Chair: James M. Smith, Centers for Disease Control and Prevention

Iodine-131 Exposure from Atmospheric Testing— The Problem and Its Significance

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From studies of persons exposed to X or γ radiation, it is well known that thyroid cancer can be induced by small doses of external radiation during childhood. In contrast, the role of ^{131}I in inducing these tumors remains unclear. Most quantitative data on cancer risks from exposure to ^{131}I come from follow-up studies of adult patients receiving exposure from diagnostic examinations and treatment for hyperthyroidism or thyroid cancer. There is little direct evidence of risk after childhood exposure to ^{131}I , and the 1994 UNSCEAR (1) report suggested that ^{131}I may be three to five times less carcinogenic than external radiation. However, recent data from Chernobyl demonstrate a remarkable increase in thyroid cancer incidence after the 1986 accident and suggest a dose-response relationship. These findings suggest that the difference in the two types of radiation may be smaller than was previously thought.

As early as 1984, Congress was concerned about the carcinogenic potential of ^{131}I exposure and passed Public Law 97-414. This law directs the Secretary of Health and Human Services to “(1) conduct scientific research and prepare analyses necessary to develop valid and credible assessments of the risks of thyroid cancer that are associated with thyroid doses of Iodine 131; (2) conduct scientific research and prepare analyses necessary to develop valid and credible methods to estimate the thyroid doses of Iodine 131 that are received by individuals from nuclear bomb fallout; and (3) conduct scientific research and prepare analyses necessary to develop valid and credible assessments of the exposure to Iodine 131 that the American people received from the Nevada atmospheric bomb tests.”

The National Cancer Institute (NCI) was requested to respond to this mandate. In so doing, a task group, established to assist the NCI in this effort, suggested that it might be possible to estimate, for each of the most important tests, the ^{131}I exposures from fallout for representative individuals and for the populations of each county of the contiguous U.S. during the time of the tests. About 100 of the tests carried out at the Nevada Test Site (NTS), with yields ranging from less than 1 kiloton to

74 kilotons of TNT, resulted in offsite detection of radioactive materials. The radiation exposures from 90 tests, representing almost 99% of the total activity of ^{131}I that had been released into the atmosphere, have been estimated and are presented in a report that was released on October 1, 1997 (2).

The most significant atmospheric weapons tests with respect to fallout occurred in the 1950s, during which time most of the monitoring of environmental radioactivity consisted of gross measurements of β particles or γ rays. Therefore, the estimation of ^{131}I exposures dating back to the 1950s was essentially derived from the original measurements of gross β -particle or γ -ray activity, or from mathematical models.

Exposures to ^{131}I in fallout resulted mainly from the pasture-cow-milk food chain. In the assessment of the ^{131}I exposures from that food chain on a continental scale, estimates were made, for each of the approximately 3,100 counties in the contiguous United States, of:

1. the activities of ^{131}I deposited on soil and vegetation,
2. the amounts of ^{131}I consumed by dairy cows and the resulting ^{131}I concentrations in cows' milk,
3. the ^{131}I ingested by people,
4. the radiation absorbed doses from ^{131}I in the thyroids of people.

Although ingestion of cows' milk is generally the predominant contributor to the intake of ^{131}I , other exposure routes need to be taken into consideration for individuals who consume little or no cows' milk. Inhalation is a minor but significant exposure route, as is the ingestion of cottage cheese, leafy vegetables and eggs. These sources are considered in the report in less detail than the ingestion of fresh cows' milk. Fresh goats' milk was a major source of ^{131}I for the small minority of people who relied upon it for a significant part of their diet. This contribution is discussed below.

For each test and each county, average thyroid doses from ^{131}I were estimated for 13 age categories, including the fetus, with adults subdivided by gender:

1. fetus (0-10 weeks; 10-20 weeks; 20-30 weeks; 30-40 weeks),
2. infant (0-3 months; 3-6 months; 6-9 months; 9-12 months),
3. child (1-5 years; 5-10 years; 10-15 years; 15-19 years),
4. adult male,
5. adult female.

For each of those age and sex groups, thyroid doses were assessed for four types and amounts of cows' milk consumed:

1. Average consumption rate of milk with the volume-weighted concentration of ^{131}I estimated for the county.
2. "High" consumption rate of milk with the highest concentration of ^{131}I estimated for the county.
3. "High" consumption rate of milk obtained from backyard cows.
4. No consumption of fresh cows' milk.

Since the publication of the report (2), the NCI has also estimated the thyroid doses that may have been received by the persons who drank goats' milk instead of cows' milk. Because the ^{131}I concentration in goats' milk is about 5 to 10 times greater than that in cows' milk for a given ^{131}I deposition on pasture grass, the relatively small percentage of the population drinking goats' milk in substantial amounts received thyroid

doses greater than those received by drinkers of cows' milk. Thyroid dose estimates for persons drinking goats' milk were made available on the World Wide Web in March 1998, where the entire report can be found (URL: <http://rex.nci.nih.gov/massmedia/Fallout>).

In addition, the per capita thyroid doses from ^{131}I have been estimated for each county and each test, each test series and all tests. The highest per capita thyroid doses for all tests, in the range from 5 to 11 cGy, are estimated not only for the stable populations in counties of states close to the NTS, like Nevada and Utah, but also for populations in counties of states relatively far away from the NTS, like Arkansas, Colorado, Idaho, Kansas, Missouri and Montana. Low thyroid doses, in the range from 0.001 to 0.1 cGy, are calculated for counties in southern California. The average per capita thyroid dose from all tests is estimated to have been about 2 cGy. Thyroid dose estimates to representative individuals vary mainly according to age, origin and consumption rate of milk, and place of residence at the time of the tests. For any particular nuclear test, the thyroid doses for children between 3 months and 5 years of age exceeded the average per capita thyroid dose after that test by a factor of about 3 to 7. Assuming that individuals in particular geographic areas consumed milk from the same source at average rates for their age group, thyroid dose estimates for young children are uniformly higher than those for adults, because of greater milk consumption and their smaller thyroid.

The potential tumorigenic effects of ^{131}I became an issue of public concern after the widespread media coverage of the NCI report. The lay public as well as public health officials wanted to know how many thyroid cancers might have been caused by the fallout—how many might have already been diagnosed and how many are predicted to occur in the future. Because direct data on ^{131}I are not sufficient for risk estimation, the predicted number of thyroid cancer cases had to be estimated based on what is known about risks from external radiation, as well as various assumptions about the carcinogenic potential of ^{131}I . The assumptions made relate to the relative biological effectiveness (RBE) of ^{131}I compared with external radiation, the shape of the dose-response relationship, the effects of gender, age at exposure and time since exposure on risk, and the projection of lifetime risks. Taking into consideration the factors mentioned above, NCI calculated that between 7,500 and 75,000 excess thyroid cancers might have occurred as a result of the fallout radiation. These estimates are subject to large uncertainties.

NCI also contracted with the National Academy of Sciences (NAS) to assess the medical and public health implications of the thyroid doses received by the U.S. population, develop policy recommendations for addressing these implications, and advise the Department of Health and Human Services on research strategies likely to help refine our risk estimates.

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