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CLOSING ADDRESS: BIOPHYSICAL ASPECTS OF AUGER PROCESSES

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I would like to begin my closing remarks by thanking the organizers of this meeting. Anybody who has ever organized an international symposium knows that this is not an easy task. The Program Committee composed of Dandamudi Rao, Kandula Sastry, and Roger Howell, and the Local Organizing Committee composed of Kandula Sastry, Roger Howell, Venkat Narra, and Michael Azure have done an outstanding job in organizing this meeting and they deserve our appreciation and a round of applause.

When I was invited to present this talk, I received precise instructions from Dandamudi Rao. He told me that my closing address should start with a comprehensive review of 20 years of radiobiological research on Auger emitters, followed by a thorough analysis of the 24 papers presented at this Symposium, and ending with overall conclusions on the current status and future prospects of the field. These were clear and explicit instructions, but when it came to preparing the talk, I started to ask myself what could possibly have prompted me to undertake such a task. It must have been an attack of egomania because it surely takes the ego of a battleship to believe that one can do what can not actually be done.

But perhaps a healthy dose of self-confidence is essential for doing scientific research. Being a scientist can be an awfully humbling experience. Nine times out of ten, when we conceive of an absolutely brilliant idea, it turns out to be wrong. That was definitely the case when I started to work on

Auger emitters in 1966. At that time I was doing cell kinetics work on cancers and I was looking for a radionuclide that could be incorporated into the DNA of cells, but would cause only minimal cell damage. So, naturally, I selected ^{125}I. With the benefit of hindsight, this obviously qualifies as one of those humbling experiences that test our self-confidence.

At about the same time Ludwig Feinendegen in Jülich also began to work with ^{125}I. I have no doubt that Feinendegen had more foresight than I and that he knew what he was doing. But with or without foresight, we both found that ^{125}I incorporated into the DNA was immensely toxic to mammalian cells. This work was soon confirmed and extended by Krisch, Adelstein, Ahnstrom, Burki, Painter, Charlton, Booz, Halpern, Warters, and other early Auger enthusiasts. But the biggest boost to the field came in 1975 when Feinendegen organized the famous Jülich meeting on Radionuclides in Biology and Medicine. This meeting brought together all the early workers on Auger emitters and recruited scores of new ones to the field.

By the late 197O's certain firm conclusions about the Auger effect started to emerge. At the risk of oversimplification, I should like to summarize in a few sentences what was known at that time.

We were aware, of course, that Auger emitters decay by electron capture and emission of a burst of Auger electrons; some of these high-energy electrons, but most of them low-energy electrons with a very short range in biological material. It was recognized from the start that emission of such a dense shower of electrons would result in a highly charged daughter atom and in a high density of electron irradiation in the immediate vicinity of the decaying radionuclide. Microdosimetry calculations from several different laboratories confirmed this conclusion, but left open the question whether the cytotoxic effects of Auger emitters were caused by high local concentrations of radiation energy or by charge effects, or possibly by a combination of both.

Regardless of the exact mechanism of action, it was obvious that incorporation of Auger emitters into radiosensitive biological structures was bound to cause extensive local damage. For example, it was demonstrated quite early that ^{125}I decays at the DNA were very efficient in inducing DNA double-strand breaks, mutations, malignant transformations, chromosome aberrations, and other types of highly localized damage in the cell genome.

At the cellular level, the effects of Auger emitters were found to depend largely on the intracellular location of the radionuclides. When incorporated into DNA, ^{125}I caused high-LET-like radiotoxic effects that exceeded those of DNA-associated ^{3}H by a factor of 15 to 25, even though the ratio of intranuclear energy deposition was calculated to be only about 2 to 4. Conversely, Auger emitters decaying outside the cell nucleus in the plasma membrane, lysosomes, mitochondria, or the general cytoplasm proved surprisingly nontoxic to mammalian cells. For example, in one experiment it took about 60 ^{125}I decays/cell at the DNA to cause 50% cell death, but when ^{125}I was attached to the plasma membrane almost 20,000 decays/cell were required to produce the same effect. Even with this massive number of decays, cell death was not caused by damage to the plasma membrane, but by overlap irradiation of the cell nucleus.

So the early work on cells quite clearly demonstrated that radiation death in mammalian cells results from damage to the cell nucleus; cytoplasmic or membrane contributions, if any, had to be minimal. By implication, these findings refuted the enzyme release hypothesis and other hypotheses that attempted to explain radiation death in terms of cytoplasmic or membrane effects rather than nuclear damage.

These studies also had important implications for medical applications of Auger emitters. It was clear that Auger emitters can be very toxic or very non-toxic, depending on where they end up within cells. The obvious conclusion was that for diagnostic applications of Auger emitters, where it is desired to keep cellular damage as low as possible, radiopharmaceuticals should remain localized outside the cell or at least outside the cell nucleus. Conversely, if Auger emitters were to be used for therapeutic applications such as radionuclide therapy of cancers, it would be necessary to find radiopharmaceuticals that can selectively, or at least preferentially, introduce Auger emitters into the nuclei of cancer cells.

So in retrospect, what we knew about Auger emitters by 1980 made a neat and appealing package. But scientists never leave well enough alone. They keep experimenting and probing until inevitably they unearth facts that no longer fit the tidy picture. Some of the more recent work, including the findings presented four years ago at the International Workshop on DNA Damage by Auger Emitters, were summarized in the opening remarks by Jim Adelstein. Without repeating Jim Adelstein's review, it is apparent that some of the research trends he identified are now coming to fruition. This is

particularly true for the growing importance of work with artificially induced Auger cascades in non-radioactive elements.

The induction of artificial Auger cascades essentially involves the use of monoenergetic X ray photons with energies either just above, or just below the K shell absorption peak of the chosen target atom. There are advantages and disadvantages to this approach. An advantage is that Auger effects can be studied on atoms for which there are no suitable Auger emitting radionuclides. Also, this method permits evaluation of the consequences of Auger showers without needing to consider the effects of atomic transmutation and other complications associated with Auger emitters. A disadvantage is the fact that large doses of external radiation must be used and it is sometimes difficult to discern the effects of the Auger cascades against the background of damage from the incident photons.

Of the 7 reports on the effects of photon-induced Auger cascades presented at this Symposium, all but one dealt with molecular aspects of radiation damage. Kobayashi (National Laboratory for High Energy Physics, Tsukuba, Japan) described the effect of K edge irradiation on cystathionine, a sulfur-containing amino acid. He reported that Auger cascades in sulfur induced very effective cleavage of chemical bonds right next to the sulfur atom, but for more distant bonds the rate of cleavage was the same with different photon energies. Takakura (International Christian University, Tokyo, Japan) examined the effects of Auger cascades originating from phosphorus atoms in adenosine-triphosphate and found that at K edge photon energies there was a 3.9-fold enhancement of adenosine-diphosphate formation, a 3.4-fold enhancement for adenosine-monophosphate, and a 1.5-fold enhancement for adenine formation. Furusawa (Tokai University School of Medicine, Japan), also working at phosphorus K edge energies, noted that Auger cascades originating at the phosphate backbone of DNA caused significant enhancement of bacteriophage inactivation.

Halpern (Kernforschungsanlage Jülich, Germany) measured DNA single-strand breaks (SSB's) and double-strand breaks (DSB's) in partially brominated plasmid DNA and observed no effects on SSB and only a minor increase in DSB from photon absorption at the K edge of bromine. He attributed the relatively small contribution of the Auger effect to the fact that the electron flux originating in light atoms outweighs that from bromine. Also working with halogenated pyrimidines were several other investigators, Nikjoo (Medical Research Council, Chilton, U.K.), Johanson (Swedish

University of Agricultural Sciences, Uppsala, Sweden), Laster (Brookhaven National Laboratories, Upton, NY, USA), and Charlton (Concordia University, Montreal, Canada). A common theme in these studies was that both bromodeoxyuridine and iododeoxyuridine incorporation into DNA enhanced the effects of ionizing radiations, with iodine producing substantially larger Auger effects than bromine.

However, these papers also point out some of the difficulties encountered in this type of work, in particular the problem of distinguishing between different mechanisms that contribute to the overall sensitization effect. Nikjoo, presenting the paper submitted by Charlton, mentioned three potential mechanisms of halogen action: (a) production of Auger electrons; (b) creation of highly reactive uracil radicals by interaction of hydrated electrons with halogenated pyrimidines; and (c) reduced damage repair in halogen-containing DNA. So we are left with the conclusion that work with photon-induced Auger cascades is very promising, but the interpretation of the results can sometimes be difficult.

Turning now to work with Auger emitting radionuclides, we again find that molecular work is becoming increasingly prominent. Haydock (Mayo Foundation, Rochester, MN, USA) studied the effects of ^{111}In decay on human carbonic anhydrase I and found that chemical transmutation from indium to cadmium was responsible for the relaxation effects (degree of molecular wobbling), but actual breaking of chemical bonds around the site of decay was caused by energy from Auger electrons.

All the rest of the molecular work dealt with the effects of Auger emitting radionuclides on DNA. Martin (Peter MacCallum Cancer Institute, Melbourne, Australia) confirmed previous evidence that the yield of DNA DSB at the site of ^{125}I decay approaches 1 DSB/decay. This finding does not support earlier work by Linz and Stöcklin (*Radiat. Res.* **101**: 262, 1985) that suggested long-range migration of excitation energy away from the site of decay. Baverstock (Medical Research Council, Chilton, U.K.) also doubts the existence of energy transfer because experiments on ^{125}I-labeled plasmid DNA do not provide any evidence for long-range energy migration in DNA. Baverstock had previously proposed solitons as a potential means of long-range propagation of excitation energy (*Nature* **332**: 312, 1988), but his experimental data did not confirm this hypothesis.

Pomplun (Forschungszentrum Jülich, Germany) presented two papers on DNA damage from Auger emitters, one using ^{123}I, the other ^{125}I. In the ^{123}I paper Pomplun examines a fundamental problem, namely the fact that after two decades of research on Auger emitters we still cannot be completely certain about the number and energies of Auger electrons emitted during electron capture decay. This has obvious implications for microdosimetry calculations. Pomplun's work also deals with another important subject matter, the relative contributions of direct and indirect effects in causing DNA damage by Auger emitters. Until recently it was assumed that essentially all the cytotoxic effects of DNA-associated Auger emitters were the result of direct effects. However, Pomplun's work, in conjunction with previous studies by Rao *et al.* (*Radiat. Res.* **124**: 188, 1990), suggests that both direct and indirect effects contribute to radiation damage by Auger emitters.

This concludes our discussion on molecular effects. Among the cellular studies there is one paper by Nagasawa (Harvard School of Public Health, Boston, MA, USA) that deals with mutation induction by ^{125}I. She reports that mutation induction at the well-studied HPRT locus varies as a function of cell cycle position, both for external X irradiation and for cells labeled with ^{3}H-TdR or ^{125}IUdR during different stages of the S phase. For all three types of exposures, mutation induction at the HPRT locus is most effective for early-S phase cells. However, X rays and radionuclides do differ in the type of mutations induced. For X rays general deletion mutants predominate, whereas for radionuclides mostly partial deletions are observed.

Turning to cellular cytotoxicity studies, DeSombre (University of Chicago, Chicago, IL, USA) describes an interesting system where estrogens are labeled with ^{123}I and used to treat estrogen receptor positive and negative cells. For this system to work the author developed an elegant method for synthesizing high specific activity ^{123}I-labeled estrogens and he finds that ER-positive cells such as CHO cells transfected with estrogen receptors are very efficiently killed in this manner, whereas ER-negative CHO cells do not respond.

Ludwikow (Swedish University of Agricultural Sciences, Uppsala, Sweden) employed ^{125}I-labeled triiodothyronine (the active form of the thyroid hormone) as a means of delivering ^{125}I to the cell nucleus. Using the production of micronuclei as an index for radiation damage, she found that although ^{125}I-triiodothyronine did preferentially accumulate in the cell nucleus of GC cells, ^{125}IUdR was about 9 times more efficient in producing cell

damage than ^{125}I-triiodothyronine. Another novel system for labeling cells with Auger emitters was described by Azure (University of Massachusetts, Amherst, MA, USA). He employed a carboplatin compound that is normally used as a chemotherapeutic agent in cancer therapy and developed a method to label this compound with the Auger emitter ^{193m}Pt. He found that this compound was efficiently incorporated into the cell nucleus of V79 cells and caused greatly enhanced cell death as compared to the cytocidal effects produced by equimolar concentrations of carboplatin containing non-radioactive platinum.

The remaining two papers in the cellular series were presented by a former student of mine, Linda Yasui, and myself. Yasui (Northern Illinois University, DeKalb, IL, USA) examined various types of DNA damage in frozen ^{125}I-labeled cells. She compared different models for the induction of DNA DSB and came to the conclusion that in contrast to the relatively clean DSB produced by X rays, ^{125}I decays produce DNA DSB composed of a whole cluster of lesions. But surprisingly, ^{125}I decay does not seem to result in the formation of DNA-protein crosslinks that are usually seen when cells are exposed to external radiations. It would appear, therefore, that this type of DNA lesion cannot play a major role in cell death. In my own paper I presented evidence that DNA-associated ^{125}I does not invariably act as a high-LET radiation source for cell killing. High-LET-type killing was seen only when decay accumulation was delayed for a few hours after cell labeling. We interpret our data as indicating that radiation death in mammalian cells may be caused by damage to higher-order structures in the cell nucleus.

In the last 5 papers we encounter a number of interesting biomedical applications of Auger emitters. Van den Abbeele (Harvard Medical School, Boston, MA, USA) describes an experimental system where ^{123}IUdR and ^{125}IUdR are used as potential diagnostic and therapeutic agents in rats and humans. After intracerebral injection of the two compounds, the radionuclides were specifically incorporated into the DNA of gliosarcoma cells and similar experiments on bladder cancer also produced positive results. The specificity of targeting was confirmed in preliminary studies on patients with colon adenocarcinoma, so it appears that radioactive IUdR may hold promise as a future diagnostic and therapeutic agent for at least some forms of cancer.

In another whole-body study, Strand (University of Lund, Lund, Sweden) examined the tissue distribution of various ^{111}In-labeled compounds

(chloride, oxine, tropolone, antibodies) in rats and found increased uptake and prolonged retention of ^{111}In in kidney, spleen, liver, bone marrow, lymph nodes, and in the testis. Within these tissues the radioactivity was preferentially incorporated into specific cell types such as macrophages in the testis. But in my opinion, the key point was the finding that ^{111}In becomes localized in the cell nucleus of certain cells. Grafström (University of Lund, Lund, Sweden), working with ^{110}In and ^{111}In demonstrated a pronounced decline in spermhead counts performed 7 weeks after administration of the radionuclides. The damage was particularly acute with X ray exposures, but both ^{110}In and ^{111}In also produced severe damage. This should raise serious questions about radiation risks from the widespread use of In-labeled compounds in diagnostic nuclear medicine.

Howell (University of Medicine and Dentistry of New Jersey, Newark, NJ, USA) examines some of the risks and problems associated with Auger emitters and α emitters by comparing the relative biological effectiveness of Auger emitters under *in vitro* and *in vivo* conditions. As expected, he finds this to be a difficult problem because the results are critically dependent on a variety of factors such as type and dose rate of the reference radiation, method of dose calculation, site of decay, and the nature of the biological end point studied. Obviously, this is an important area of research and additional data are needed.

Finally, in the last paper of the Symposium, Narra (University of Medicine and Dentistry of New Jersey, Newark, NJ, USA) revisits the question as to whether or not the cytotoxic effects of Auger emitters can be counteracted by treatment with chemical radioprotectors such as cysteamine. Using spermhead counts as the assay system, he finds that cysteamine provides protection against both α emitters and Auger emitters, but the degree of protection is much higher for Auger emitters. He suggests that the Auger effect may be largely due to indirect action from radicals which certainly is radically different from what at least I would have predicted.

This concludes my review of the unusually interesting papers presented at this Symposium and brings me to the final part of my remarks, my predictions for the future. But before you reach for your pencils to write down all the sage advice, please remember my record as prognosticator and how I came to work with ^{125}I in the first place. Not everybody can be as gifted in handing out advice as Conrad Hilton, the legendary founder of Hilton Hotels. When asked what words of wisdom he had for the world he replied,

"whenever you take a shower, always put the curtain inside the tub." This is phenomenally good advice, especially if you happen to be the owner of a hotel chain where half of the guest fail to follow this important rule.

I have nothing of such moment to contribute today, but a few modest thoughts might be worth mentioning. One emerging trend in our field is the increasing emphasis on medical applications of Auger emitters, both in diagnostic and in therapeutic nuclear medicine. For many years large numbers of different Auger emitters have been used for diagnostic applications and the situation is further complicated by the fact that these radionuclides are administered in a great variety of chemical configurations. So the problem of assessing risks is by no means trivial and will require considerable additional experimental and theoretical work. At the same time, Auger emitters hold great promise in radionuclide therapy of cancers if (and that is still a very big IF) suitable methods for selective or at least preferential radionuclide delivery to cancer cells can be developed. As pointed out by Feinendegen, "molecular surgery" by Auger emitters could one day provide a means of realizing Paul Ehrlich's dream of a "magic bullet" in cancer therapy.

Equally important will be the continuation and expansion of basic molecular and cellular studies on the Auger effect. Radiation exposure of cells induces mutations, division delay, malignant transformation, premature aging, and outright cell death. The mechanisms and targets for these effects are still not known and should be further analyzed with Auger emitters. For example, based on work with ^{125}IUdR incorporated into DNA, Schneiderman *et al.* (*Radiat. Res.* **116**: 283, 1988) concluded that the primary target for division delay may not be the DNA, but a so far unidentified non-DNA target located at or near the nuclear envelope. Whether or not this structure contributes to radiation-induced cell death or other types of cellular radiation damage remains unknown.

Cancer induction is another example where work with Auger emitters might answer fundamental questions. It is generally agreed that malignant transformation is a two-stage process consisting of an initiation phase and a promotion phase. Initiation is believed to result from damage to DNA, but promotion is almost certainly an epigenetic process that somehow permits or induces the expression of malignant properties in initiated cells. Radiation acts both as an initiator and promotor, so work with Auger emitters incorporated into different subcellular locations might permit us to more

clearly distinguish between these two phenomena and perhaps also to identify the so far unknown target for promotion.

Radiation is also known to accelerate the process of aging both in cells and in whole organisms. Again, in spite of numerous conflicting hypotheses, virtually nothing is known about the molecular and cellular mechanisms responsible for aging. As far as I am aware, no aging work has been performed with Auger emitters and I would not be surprised if studies along these lines were to produce novel insights into aging processes.

These are only a few examples of potential future directions and I am sure that everyone in this audience could add additional possibilities. All these applications depend on the unique decay and dose distribution characteristics of Auger emitters which permit differential radiation exposures of subcellular and even submolecular sites. In many ways, the difference between Auger emitters and external radiations is analogous to the difference between smart bombs and conventional bombs. Auger emitters act like smart bombs that destroy individual components of the system, with only minor collateral damage to distant sites. In contrast, exposure to external radiation is comparable to large-area carpet bombing that indiscriminately damages the system as a whole. Dave Charlton, who dislikes modern warfare, prefers a classical military analogy and compares the Auger effect to a Trojan horse. As long as the horse remains outside of Troy it is harmless, but when brought into the city the consequences can be fatal.

Whether you regard Auger emitters as smart bombs or Trojan horses, there can be no doubt that they offer the potential for unique contributions to our understanding of radiation action. Louis Pasteur once wrote that "all things remain hidden, obscure, and undebatable as long as the cause of the phenomena be unknown, but everything becomes clear when this cause be known". Judging from the work presented at this Symposium we can be fairly confident that when we meet again in 1995 in Sweden, some of the causes that are now obscure will be clear, and Auger emitters will have played an important role in illuminating these causes.