RISK AT LOW DOSES: SCIENTIFIC KNOWLEDGE, UNCERTAINTIES AND MANAGEMENT.

Augusto Giussani, Francesca Ballarini,

Università degli Studi di Milano, Italy

Andrea Ottolenghi

Università degli Studi di Pavia, Italy

1. RISK AT LOW DOSES:

Most of the applications of ionizing radiation in the medical field, for the exposed workers as well as the majority of patients undergoing diagnostic examinations, can be seen as low dose situations. Epidemiological information is however available for dose and dose rates higher than the values typical of most medical situation.

Main source of information is the Life Span Study (LSS) of Japanese A-bomb survivors, supplemented by studies of selected groups of exposed workers (uranium miners, radium painters) or radiotherapy patients with a detailed follow-up history. All of these group studies, however, suffer from one or more of the following limitations:

- lack of adequate dosimetry,
- lack of a reliable control group for the necessary comparison,
- influence of concomitant factors (not always easy to find out)
- influence of social conditions.

In addition, exposed study populations are different than the population of patients for which the risk estimates are needed in the medical situation. Recent studies aimed to evaluate the available data on the cohorts of the inhabitants of the Techa river settlements as well as of the workers of the Mayak nuclear facilities may provide in the future useful information on large populations chronically exposed to relatively low doses.

For the time being, however, the difficulty in having clear and reliable epidemiological indications at low levels of exposure makes it necessary to introduce assumptions about the related risks. It is commonly assumed (UNSCEAR 2000, ICRP 1991) that the risks for low LET radiation at low doses can be extrapolated from data at higher doses considering a reduction factor (DDREF, set equal to 2 in the ICRP 1990 Recommendations). Moreover, the so called linear-no threshold (LNT) hypothesis has been adopted. According to this assumption, the risk at low doses is linearly proportional to the dose, and the absence of a threshold determines that any exposure, no matter how low, must be associated with a risk.

The linear assumption enables one to sum the risks due to exposures received at different times and/or from different sources. This fact is very practical in the management of the radiation protection of exposed workers, since it simply requires to monitor the cumulative dose of the exposed subject and compare it to limits that have been set on the basis of the related risks. On the other side, the absence of a threshold requires that any possible source of exposure should be individuated and any protective measures considered, taking into account economical and social factors. Of course the overestimation of risks at very low doses may play a crucial role in balancing risks and benefits in particular situations, especially when economical factors enters into play.

With reliable epidemiologic data at low doses not available for the reasons mentioned above, biological research can supplement fundamental information on the mechanisms of radiation effects. In the recent years, a series of investigations have put into question the validity of the LNT assumption, although the conclusions that can be drawn seem to be contradictory.

Two phenomena, in particular, are being investigated with great interest, as developments in experimental facilities and analytical techniques make it possible to perform radiobiology studies at low exposure levels: on one side are the so-called *bystander effects* (BE), on the other side the *adaptive response* (AR).

The name *bystander effects* indicates the induction of damage in cells located in the vicinity of irradiated cells, but that have not been directly hit themselves. Bystander effects depend on different factors, like:

- the radiation quality,
- the dose and dose-rate,
- the treatment type,

- the cell culture condition,
- the cell line,
- the observed end-points (clonogenic activation, apoptosis and micronuclei, mutations, gene expressions, chromosome aberrations, transformation…).

Some significant results are here briefly summarized. For example, Nagasawa and Little (1992), have irradiated cultured cells with _ particles and doses between 0.03 and 0.25 cGy, so that only about 1% of the cells was traversed by the incident particles, and have observed sister chromatide exchanges in more than 30% of the population. Experiments by Azzam et al. with _ particles (2001) have indicated that the expression of genes like *p53* and *p21*, involved in the processes of cell-cycle controls and in the induction of apoptosis, were modified in a non-linear way after low-dose irradiation. Other studies on the induction of cell inactivation (Sawant et al., 2001) and of gene mutations (Zhou et al., 2001) have demonstrated that the same results were produced by targeting 10% or 100% of the cell population.

Most of the studies on BEs were made possible by the availability of microbeams, able to direct very low numbers of incident projectiles (light ions) onto single cell nuclei. Such experiments are more difficult to conduct with sparsely ionizing radiation like photons: the modality of interaction and of delivery of energy to the target makes it more difficult to design experiments in which only a proportion of the cells is hit. However, some low-energy x-ray microbeam facilities are available (e.g., at the Gray Laboratories in the UK) and experiments are ongoing.

Damage in non-hit cells can be explained considering the possibility that cells communicate and transmit information with one another. The mechanisms employed by the cells for exchanging the information about radiation damage are still unclear. However, intercellular communication via gap-junctions (narrow channels of approximately 2 nm diameter that connect the cytoplasms of two adjacent cells and allow diffusion of small molecules) seem to play an important role, and the cell signalling can be triggered (at least in some cases) by the reactive oxygen species (ROS) produced by the action of the ionizing radiation.

Interestingly, essential information on the modality of signal transmit was obtained by investigations making use of the treatment with irradiated conditioned medium ("ICM treatment"). In these studies (Mothersill and Seymour, 1998 and 2001, Seymour and Mothersill 1999) non-irradiated cells were fed with the medium of culture cells previously irradiated, and the result was a decrease in the cell survival also in the non-irradiated population. Experiments conducted by irradiation of the medium alone did not produce, on the contrary, any significant difference with the control population, indicating that free radicals produced by hydrolisis of medium components do not play any role in this context. It seems therefore that hit cells can release into the medium a factor able to affect the survival of the non-exposed cells, at least of those of the same line. This factor has been identified by some authors in the protein interleukin-8 (Desphande et al., 1996, Narayanan et al., 1999). The dimensions of protein-like signalling agents are however too large for being consistent with the assumption of gap-junction intercellular communication.

The interpretation of the *bystander effects* may be different with regard to the end-point considered. If the induction of transformations and/or mutations is taken as parameter (Nagasawa and Little 2001, Sawant et al. 2001, Zhou et al., 2001), then it can be argued that the risks at low doses are higher than resulting from a linear dose-effect relationship. As a consequence, greater importance should be given to the optimization process, in order to avoid any undue exposure which may be more harmful than previously thought. On the contrary, the increased ratio of apoptosis (Belyakov et al., 2000, Prise et al., 2000) suggests a possible protective effect, considering that potentially damaged cells are removed from the surviving population. The impact of assuming a possible protective action on the management of low-dose exposures will be considered in detail in the following discussion on the *adaptive response*.

This phenomenon can be described as the activation and stimulation of cell defence systems (like, for example, the DNA damage repair mechanisms and antioxidant activities) after administration of a low radiation dose (of the order of few cGy), so that the organism can fight with greater efficiency a subsequent "attack" from a cancerogenic agent, not necessarily of radiative nature (Wolff, 1998, Feinendegen, 1999).

Mitchel and Boreham (2000), for example, demonstrated that the efficacy of repair of chromosomal aberrations after exposures to doses of 1 Gy and greater, was increased in cell populations previously exposed to low and moderate doses (going from a few cGy up to 0.5 Gy) delivered at low dose-rate. In addition, also the frequency of malignant transformation was observed to have been reduced as effect of an adaptive pre-irradiation at low doses (Azzam et al., 1994). It was also demonstrated that spontaneous malignant transformations were reduced after irradiation at low doses from 1 to 100 mGy (Azzam et al., 1996). Positive results were obtained not only in cell populations, but also in laboratory animals (Mitchel et al. 1990, 1999). The latency period before appearance of myeloid leukemia in mice increased as a consequence of the adaptive dose. Interestingly, this mechanism seems to be effective not only against a subsequent radiation offence, but also to protect against tumours induced by chemical agents (Mitchel et al. 1990).

The afore-mentioned results are in sharp contrast with the LNT-hypothesis. If the linear assumption were valid, then the effects of the preliminary (low dose) irradiation and of the subsequent (high-dose) exposure should sum up. But this is evidently not the case: the total risk is clearly even less than each of the single risks taken separately. In addition, the possibility of a positive effect of low-dose/low-dose-rate exposures (hormesis) cannot be excluded (Azzam et al., 1996, Macklis and Beresford, 1991, Mine et al., 1990). The mechanisms of cellular communication seem to be able to explain also the evidences of AR; in this case, intracellular communication could play a key role.

Should these evidences be taken into account, then the whole principle of optimization of radiation protection would gain a different meaning. The same ALARA principle would no more be valid, because in this case the reduction of doses to values as low as possible would not mean necessarily a lower risk. In particular, the re-evaluation of the risks would lead to a different balance between risks and benefits, which would determine a new perspective with regard to the justification of given procedures. A simple example may be the decomissioning of nuclear facilities or the decontamination of accident sites, where considerable amounts of money are spent to achieve levels of residual activity which are considered safe under the current assumptions. Probably much of this work could result superfluous, if evidence is accepted that low exposures are not harmful. The statement by Mitchel and Boreham (2000) that "… it may be imprudent and incautious not to expose radiation workers to low doses of radiation, when there is a possibility that such exposures, while doing no harm, will protect them against the known harmful effects of higher doses to which they might later be exposed….." can be considered a little exaggerated, but probably could be a stimulus for further studies and discussion.

Both the *bystander effects* and the *adaptive response* seem therefore to question the validity of the LNT-hypothesis, although with different consequences. The apparent contradictions between these two phenomena may be traced back to the different LET of radiations (mainly light ions for BE, electromagnetic radiation for AR), to the targets (cell populations, cultured tissues, animal models) and/or to the end-points considered (in some cases there is no direct relationship between the end-points studied and the eventual induction of malignancies). Analysis of the epidemiological data, mainly of the LSS of the A-bomb survivor, are also controversial, as they seem to indicate in some cases a linearity between dose and risk also at low doses, but cannot exclude a threshold at 60 mSv. (Pierce and Preston, 2000, Little and Muirhead, 2000). Studies of populations living in areas with anomalously high (or low?) background radiation levels could probably provide additional useful information.

Summarizing, the data available are interesting but inconclusive, and the discussion in the scientific world is open and vibrant, also in consideration of the impacts deriving from a change in the philosophy with which the question of low-dose exposures is addressed. Further research should be therefore encouraged and stimulated in order to shed more light on the controversial issues, and it should be desirables to have more works that can be directly compared in terms of experimental design and end-point considered. The development of mathematical models can be crucial for the comprehension and description of the mechanisms involved; for a more correct interpretation of the experimental data available the interaction of the cells with their immediate environment should be considered (Ballarini et al., 2002).

With so many questions still unanswered (and so to remain in next few years), there is evidently no indication that ICRP will make any change to the LNT-hypothesis in its new Recommendations intended to be issued around 2004 (Clarke, 2000, 2002). The optimization of protection will therefore continue to be in the sense of reducing the exposures to levels as low as reasonably achievable, since this is considered to maximize the margin between benefit and harm.

In the case of the medical field, two aspects must be considered. One is that the ever-growing attention to the radiological protection of the patients in the recent years (IAEA, 2001) will surely have a positive effect also on the protection of the workers in the medical field. A second aspect, directly linked to the first one, is that there is still room for optimization of medical exposures.

Often the justification of the medical practice and the benefits expected for the patients have been seen as sufficient to manage exposures. It must kept in mind that of course the primary aim of the medical application of radiation is to obtain a diagnostic or therapeutic results, and that dose cannot be reduced indefinitely without prejudicing the desired outcome. However, a simple example taken from the recent UNSCEAR Report (UNSCEAR 2000) shows that a better management of exposures is possible. If we consider patients undergoing a common diagnostic chest radiography in some European countries (Table 15, Annex D of the UNSCEAR Report), we find out that doses may vary from 0.02 mSv per procedure in the UK to 0.1 mSv pp in Switzerland and in Finland, 0.13 mSv pp in Norway, 0.15 mSv pp in Sweden, and 0.3 mSv pp in Germany. That means that there is a factor up to 15 of difference between industrialized countries, all considered to have an advanced system of medical assistance (Health Care Level I). So greater efforts should be concentrated in optimizing procedures in order to be able to obtain reliable diagnostic information with the minimum of exposure (to the patients as well as to the personnel).

The use of diagnostic reference levels has often been considered as an indication of good practice, whereas it should be more appropriate to consider the exposures exceeding such levels as an alarm that should trigger investigations on the underlying reasons. At the same time, advances in technology and safety practices should be taken into account in order to continuosly improve and optimize the diagnostic procedures, unless the improvements are too costly or unnecessary complex. This should result in a constant redction to lower values of the reference levels. It should also be observed that the improvement and optimization of protection can result in the justification of a practice that was previously unjustified due to excessive doses.

However, a major factor of concern in the medical field is the ever growing use of ionizing radiation in a number of new practices, like for example interventional radiology (ICRP, 2001). These procedures are often characterized by moderate to high dose levels both to the personnel and to the patient. In the majority of cases, these interventions are conducted by medical doctors (e.g. cardiologists, urologists, surgeons….) who do not have a specific training on radiation, are not aware of the related risks and of the simple methods for reducing occurrence of injuries.

An adequate training in radiation safety and management is therefore essential for all clinicians, not only for those specialists traditionally involved with radiation. It seem therefore desirable that all medical students are provided with an introductive course in radiation protection and safety already at the beginning of their academic studies, and that this issue be also considered in the programs for continuos training and education of all specialists. An appropriate training should also be given to the general referring physicians, with whom the patients have the first and most frequent contacts. They should be made aware not only of the most recent advances in the diagnostic technologies, but also of the related risks and of possible valid alternatives that do not make use of ionizing radiation. The use of a personal patient identification card, with indications of all exposures to radiation, could be of help in order to avoid unnecessary repetitions of basic diagnostic investigations.

On the other end, physicists and radiation protection officers should keep the pace with the technological and commercial progresses, in order to be able to provide an updated protection for both staff and patients. Like for the medical doctors, programs for training and continuous education should be made available on a regular basis. In addition, it must be considered that many of the the most recent applications (i.e., interventional radiology in cardiology) can be life-saving procedures, and that any protective measure should not prevent them from achieving the desired effect. A close cooperation between medical doctors and radiation safety experts, with mutual exchange of information about needs and constraints, is therefore a necessary requirement for an optimal management of the medical exposures.

Of course adequate resources should be made available for the conduction of the training programs. Particular attention should also be paid to the regular update of the contents, as well as to the qualifications of the personnel in charge of these programs ("training of the trainers").

In conclusion, it is possible to say that the basic research still needs time and resources to clear the issues of exposure to low doses. Whichever is the relation between doses and risks, however, it is evident that the management of exposures in the medical field will always require a continuous and serious education of all professional figures involved, and mutual cooperation and understanding.

2. **LIST OF REFERENCES:**

Azzam E.I., Raaphorst G.P. and Mitchel R.E.J. (1994): *Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10t_ mouse embryo cells,* Radiat. Res. **138**, S28-S31.

Azzam E.I., de Toledo S.M., Raaphorst G.P. and Mitchel R.E.J. (1996): *Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10t_ cells,* Radiat. Res. **146**, 369-73.

Azzam E.I., de Toledo S.M. and Little J.B. (2001): *Direct evidence for the partecipation of gap junction-mediated intercellular communication in the transmission of damage signals from α-particle irradiated to nonirradiated cells*. Proc. Natl. Acad. Sci. USA **98**, 473-8.

Ballarini F., Biaggi M., Ottolenghi A. and Sapora O. (2002): *Cellular communication and bystander effect: a critical review for modelling low-dose radiation action*. Mut. Res. **501**, 1-12.

Belyakov O.V., Prise K.M., Mothersill C., Folkard M. and Michael B.D. (2000): *Studies of bystander effects in primary uroepithelial cells using a charged particle microbeam*. Radiat. Res. **153**, 235.

Belyakov O.V., Malcomson A.M., Folkard M., Prise K.M. and Michael B.D. (2001): *Direct evidence for a bystander effect of ionizing radiation in primary human fibroblasts*. BJC **84**, 674-9.

Bennett B., Bouville A., Hall P., Savkin M. and Storm H. (2000): *Chernobyl accident: exposures and effects*. Tenth International Conference of the International Radiation Protection Association IRPA-10, Hiroshima (Japan).

Brenner D.J., Little J.B. and Sachs R.K. (2001): *The bystander effect in radiation oncogenesis: II. A quantitative model*. Radiat. Res. **155**, 402-8.

Cai L. (1999): *Research of the adaptive response induced by low-dose radiation: where have we been and where should we go?* Hum. Exp. Toxicol. **18**, 419-25.

Clarke, R.H. (2000): *Progress toward new recommendations from the international commission on radiological protection*. Tenth International Conference of the International Radiation Protection Association IRPA-10, Hiroshima (Japan).

Clarke R.H. (2002): *Radiological protection phylosophy for the 21st century. Workshop on internal dosimetry of radionuclides*. Occupational, public and medical exposure. Oxford (UK).

Deshpande A., Goodwin E.H., Bailey S.M., Marrone B.L. and Lehnert, B.E. (1996): *Alpha particle-induced sister chromatid exchange in normal human lung fibroblast: evidence for an extranuclear target.* Radiat. Res. **145**, 260-7.

European Commission (2001). *Radiation protection 125: Low dose ionizing radiation and cancer risk*. Office for official publication of the European Communities, Luxembourg.

Feinendegen, L.E. (1999): *The role of adaptive responses following exposure to ionizing radiation*. Hum. Exp. Toxicol. **18**, 426-32

IAEA (2001). International Conference on Radiological protection of patients in diagnostic and interventional radiology, nuclear medicine and radiotherapy. IAEA CSP 7/CD.

ICRP (1991). *Publication 60: 1990 Recommendations of the International Commission of Radiological Protection*. Elsevier, Oxford.

ICRP (1996). *Publication 73: Radiation protection and safety in medicine*. Elsevier, Oxford.

ICRP (2000). *Publication 85: Avoidance of radiation injuries from medical interventional procedures*. Elsevier, Oxford.

Ikushima T., Aritomi H. and Morisita J. (1996): *Radioadaptive response: efficient repair of radiation-induced DNA damage in adapted cells*. Mutat. Res. **358**, 193-8.

Little M.P. and Muirhead C.R. (2000): *Derivation of low-dose extrapolation factors from analysis of curvature in the cancer incidence dose response in Japanese atomic bomb survivors*. Int. J. Radiat. Biol. **76**, 939-53.

Macklis R.M. and beresford B. (1991): *Radiation hormesis*. J.Nucl. Med. **32**, 350-9.

Mine M., Okomura Y., Ichimaru M., Nakamura T. and Kondo S. (1990): *Apparently beneficial effect of low to intermediate doses of A-bomb radiation on human lifespan*. Int. J. Radiat. Biol. **58**, 1035-43.

Mitchel R.E.J., Gragtmans N.J. and Morrison D.P. (1990): *Beta-radiation-induced resistance to MNNG initiation of papilloma but not carcinoma formation in mouse skin.* Radiat. Res. **121**, 180-6.

Mitchel R.E.J., Jackson J.S., McCann R.A. and Boreham D.R. (1999): *The adaptive response modifies latency for radiation-induced myeloid leukemia in CBA/H mice.* Radiat. Res. **152**, 273-9.

Mitchel R.E.J. and Boreham D.R. (2000): Radiation protection in the world of modern radiobiology: time for a new approach. Tenth International Conference of the International Radiation Protection Association IRPA-10, Hiroshima (Japan).

Mortazawi S.M.J., Ikushima T., Mozdarani H. and Sharafi A.A. (2000): *Synergistic effect versus radioadaptive response: possible implications of such a response in the estimation of risks of low-level radiation can be more problematical*. Tenth International Conference of the International Radiation Protection Association IRPA-10, Hiroshima (Japan).

Mothersill C. and Seymour C. (1998): *Cell-cell contact during gamma irradiation is not required to induice a bystander effect in normal human keratinocytes: evidence for realease during irradiation of a signal controlling survival into the medium*. Radiat. Res. **149**, 256-62.

Mothersill C. and Seymour C. (2001): *Radiation induced bystander effect: past history and future directions*. Radiat. Res. **155**, 759-67.

Nagasawa H. and Little J.B. (1992): *Induction of sister chromatid exchanges by extremely low doses of alpha particles*. Cancer Res. **52**, 6394-6.

Narayanan P., LaRue K.E.A, Goodwin E.H. and Lehnert B.E (1999): *Alpha particles indue the production of interleukin-8*. Radiat. Res. **152**, 57.

Pierce D.A. and Preston D.L. (2000): *Radiation-related cancer risks at low doses among atomic bomb survivors*. Radiat. Res. **154**, 176-86

Prise K.M., Belyakov O.V., Malcomson A.M., Ozols A., Folkard M. and Michael B.D. (2000): *Understanding bystander responses in cellular systems: studies using a charged particle microbeam*. Radiat. Res. **153**, 235.

Sawant S.G., Randers-Pehrson G., Geard C.R., Brenner D.J. and Hall E.J. (2001): *The bystander effect in radiation oncogenesis: I.Transformation in C3H 10 T_ cells* ***In Vitro*** *can be initiated in the unirradiated neighbors of irradiated cells*. Radiat. Res. **155**, 397-401.

Sawant S.G., Randers-Pehrson G., Metting N.F. and Hall E.J. (2001): *Adaptive response and the bystander effect induced by radiation in C3H 10 T_ cells in culture*. Radiat. Res. **156**, 177-80.

Seymour C. and Mothersill C. (1999): *Cell communication and the "bystander effect"*. Radiat. Res. **151**, 505.

Shigematsu I. (2000): *Lessons from atomic bomb survivors in Hiroshima and Nagasaki*. Tenth International Conference of the International Radiation Protection Association IRPA-10, Hiroshima (Japan).

Streffer Ch. (2000): *Genetic predisposition and implications for radioprotection*. Tenth International Conference of the International Radiation Protection Association IRPA-10, Hiroshima (Japan).

UNSCEAR (2000): *Sources and Effects of Ionizing Radiation: United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) 2000 Report to the General Assembly with Scientific Annexes*. United Nations Publications Sales No. E.00.IX.6, New York

Wolff S. (1998): *The adaptive response in radiobiology: evolving insights and implications*. Environ. Health Perspect. **108**, 277-83.

Zhou H., Suzuki M., Randers-Pehrson G., Vannais D., Chen G., Trosko J.E., Waldren C.A. and Hei T.K. (2001): *Radiation risk to low fluences of* α *particles may be greater than we thought*. Proc. Natl. Acad. Sci. USA **98**, 14410-5.