Low level radiation exposure
The radiobiologist’s challenge in the next millennium*

Abraham F G Stevenson
Institute for Toxicology, Centre for Environmental Sciences, University of Kiel
Brunswiker Str. 10, 24105 Kiel, Germany

A formal definition for low level exposure does not exist. This has arbitrarily been defined here as exposures from 0 to 5 cGy. The health implications of exposures within this dose range are highly controversial since the effects are exclusively stochastic. As such, the effects can only be detected in large populations. The Oxford Survey of Childhood Cancers (OSCC) established leukaemia as a predominant effect. After the Chernobyl nuclear disaster, studies in European countries have correlated perinatal mortality with radioactive contaminations which could only have raised the radiation burdens by levels which are currently regarded as negligible. The reported risk indices for childhood leukaemia arising from low level exposures are generally comparable to those ascertained for high exposures, thus posing an enigma to radiobiologists. This paper reviews the progress in various areas of radiobiological research and attempts to make a synthesis of the facts with the view to provide an explanation. The purpose is also to stimulate an understanding of multifactorial biological mechanisms. Environmental radiation exposures must be expected to be concomitant with other toxic agents which must be taken into account in risk assessment. The challenge in the future will be to realise this goal.

There is no consensus of opinion in the international community of radiation biologists as to what low dose or low level of exposure to ionising radiation is. Low and high are relative terms which depend on perception. At about the time when the forebearer of the present ICRP (International Commission for Radiological Protection) was founded (in the mid-twenties)-in recognition of the dangers of ionising radiations-annual exposure to 1.6 Sv X-rays was considered safe. Since then downward corrections of the maximum permissible annual dose for occupationally exposed persons has taken place, until the ICRP (1958) came up with the concept of the “genetic dose” which was the best estimate of the doubling dose per generation (set at 30 years). This dose (5 cSv) was recommended as the maximum permissible annual dose for occupationally exposed persons¹, which has since been observed in most countries. The annual dose limit to the general population has been derived from this value, by taking a thirtyth fraction and an additional safety margin. The practice can vary between countries. Revisions by the ICRP have followed in 1990 for occupationally exposed persons² and in 1997 for the general population³. The former has been set at 2 cSv/a and the latter ought to be optimised from 0.1 cSv/a to 0.03 cSv/a. Radiation exposures at these levels are generally regarded by radiobiologists as negligible, although the currently accepted view on the stochastic effects of ionising radiations is a linear relation to dose passing through the origin. Only leukaemias are thought to deviate from this dose relation, by having an exponential component at higher doses.

Conservative scientific bodies have upheld the opinion that the approximate borderline at which direct evidence of stochastic effects has been proven beyond doubt, is about 50 cGy. They do concede that there may be evidence of such effects at doses down to 5 cGy. Exposures below this dose level are subject to doubt, since the kind of studies needed to produce cogent evidence would be exceedingly large. It should be mentioned in this context that recent data from the RERF (Radiation Effects Research Foundation, Japan) indicates significant cancer mortalities for the dose group 0 - 10 cGy.⁴ Thus the dose range between 5 and 50 cGy has been tacitly considered as low dose exposure, and deliberations on effects at exposures below 5 cGy have been regarded as being based more on speculation than on facts. This is, of course, inconsistent with the currently largely accepted

* Dedicated to Otmar Wassermann, DSc, Professor emeritus, University of Kiel, toxicologist par excellence and unrelenting champion against chemical and radioactive pollution of the environment.
concept of a linear dose-effect relationship, with no threshold for stochastic effects.

Low radiation doses will be regarded here as exposures above the natural background radiation burden, the upper limit being doses at which stochastic effects are more readily detectable. It is also at this upper limit that subtle cellular effects are just measurable. This upper limit could be somewhere about 5 cGy. The intervening dose range between 5 and 50 cGy could be designated as low intermediate and doses from 50 cGy to 1 Gy as high intermediate. All doses above 1 Gy may be designated as high doses, since deterministic effects begin to manifest. This classification is of course arbitrary. Low dose exposure is that area of radiation biology which bears few definitive data, being restricted by the very nature of the problem. Risk predictions for stochastic effects have depended on the retrogressive extrapolation of the RERF data from the intermediate and high dose ranges. Since the RERF data spans over a period of about 50 years, the latencies for most tumours have been attained. Although cancers are still recorded - particularly for the groups which received 10 cSv or less - the peaks for the occurrence of most cancers have been surpassed. The ongoing collection of data from this dose group will strengthen the accuracy of extrapolation to the low dose levels.

On account of the lack of sufficient firm data, the best fit for the back-extrapolation to low doses is still controversial. Based on scanty human data and experimental observations, five models have been postulated6.7. As shown in Fig.1, these models provide the basis for risk estimations at low doses. The model that presently receives the greatest general acceptance is the linear model (Fig. 1A) which passes through the origin. It is the current working model employed by international bodies especially for solid tumours. The other model which is favoured by BEIR (Committee on the Biological Effects of Ionising Radiations, National Academy of Sciences, USA) is linear-quadratic (Fig.1B), essentially curvilinear and concave - and is applied to leukaemias. Two models which no longer receive support in mainstream radiobiology are the one which claims that a threshold exists for stochastic effects i.e. no effects are discernible up to a certain dose level, after which the correlation is linear (Fig.1C). The other currently discredited model goes a step further in its claim that a limited amount of low level exposure is beneficial to health (hormesis): the curve, therefore, takes an initial dip below zero and re-crosses the abscissa, pursuing a linear course thereafter (Fig. 1, D). The last model which is also the most recent, has not received attention in mainstream radiobiology (Fig. 1, E)7. It takes a course which is practically the opposite of the afore-mentioned linear-quadratic model in that the quadratic component is at the beginning. The curve is accordingly convex and has been described as a supra linear model by the authors i.e. at very low doses, the effect is higher than what would be expected from a linear relationship.

The question at stake is whether low level radiation exposure, as defined in this paper, poses health risks which are above expectation. Since only stochastic effects are of relevance at low doses, and the fact that current understanding foresees the absence of any threshold, answers the question partially, i.e. exposures however small must have effects. To demonstrate such effects requires exceedingly large collectives of persons. The crux of the issue is whether current risk estimates represent substantial underestimations. An enigma in radiation biology has been the rift between knowledge accrued from experimental work and findings from epidemiology. Epidemiologists have consistently established health risks at low dose exposures (as defined here), which experimentalists consider quite safe. To make matters worse, the indices of health risks ascertained by

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Fig. 1—Existing models on the dose-response at low levels of exposure. A: Linear model B: Linear-Quadratic model C: Threshold (T) model D: Hormesis model E: Supralinear model.
epidemiologists fluctuate at comparable levels for both low and intermediate doses, suggesting that low doses are more efficacious. However, current experimental studies are bringing forth novel findings which may facilitate insight into the biological mechanisms involved, in order to explain the phenomenon. It is the intention here to sketch relevant developments and to provide an answer to the question as to whether low levels of exposure to ionising radiations pose health risks.

**Leukaemia: A pre- eminent indicator of radiation insult**

The somatic stochastic effects of exposure to ionising radiations are expressed as cancers. Ionising radiations are known to be unspecific in the kinds of cancers induced. In other words, they are able to induce any of the known tumours/cancers. It is for this reason that ionising radiations have been a favourite tool in trying to understand the mechanism(s) in cancerogenesis, as it is thought that radiation induced cell transformation is none other than the blue print of naturally occurring processes. From the time of induction until detectability or visible expression of disease, varying lengths of time can elapse - latency periods - depending on the type of tumour/cancer and other biological factors like age at the time of induction. The latency period for leukaemias is generally the shortest especially for childhood leukaemias. The RERF data on the atom bomb survivors indicate that the latency peaks about the fourth or fifth year in children and is about double in adults. In cases of infant leukaemias, induction may be expected to have occurred in utero. This is a currently accepted concept, although evidence was first brought forward by the Oxford Survey of Childhood Cancers (OSCC) conducted by Alice Stewart8-10 in the United Kingdom four decades ago. Since the RERF data forms the bulwark of radiation cancer epidemiology, it has served as the cornerstone for the recommendations made by the ICRP, despite a serious drawback in the data basis due to the loss of the first five years of mortalities. These lost cases represent the weakest members of the cohort i.e. the youngest children and the aged, as testified by an analysis of the age profile of the cohort11. This loss may be partially compensated for through the data published by Folley et al12. Various forms of cancer excesses are being recorded among the living survivors right to the present day.

The radiation exposure of the RERF cohort is attributed to external exposure from the atomic blast. The doses which were received by the population ranged from the low intermediate, through the high intermediate, right up to sublethal high doses. Other data from experimental animals or from epidemiological studies also mostly involve external exposure. The use of these data for risk estimates in situations where radionuclide incorporation is involved, is inappropriate. Human experience arising from radionuclide incorporation has come from retrospective studies on radium dial painters, thorotrast patients and uranium miners who were exposed to high levels of radon in the mines. Since the dose estimates are bound with large uncertainties, derived risk estimates must be viewed with due caution. Animal experimental studies on cancer induction after the incorporation of bone seeking radionuclides (strontium-90, radium-226 and plutonium-239) are very limited, and as far as leukaemia is concerned, even controversial. Taken at surface value, the gist of all these studies indicate that alpha emitters are poor inducers of leukaemia, but highly effective inducers of liver cancers (plutonium-239) and osteosarcomas (both radium-226 and plutonium-239). The obvious reason for low leukaemogenesis is the predominance of cell killing. The cardinal question which has not been answered is whether the leukaemogenic potential can be stepped up through the reduction of cell killing by sheer dose reductions i.e. less incorporation. However, the problem is highly complex because of the all-or-none effect of alpha irradiation. Exposed tissues consist of unhit and lethally damaged cells, so that dose reductions simply result in shifting the proportions of the two. It is thus difficult to imagine that manipulations in this manner might influence the leukaemogenic potential. Recent experimental studies have come up with various biological concepts which are removed from physical mechanisms, and these will be addressed later in this context.

Being a rare disease, the risk for leukaemia expressed in numbers of persons will always be small compared to solid tumours. Owing to the sensitivity of haemopoietic cells to ionising radiation, the doubling dose for leukaemias is exceedingly low. This coupled with the relatively short latency period, makes this group of diseases a cardinal indicator for radiation exposure. Taken as a whole, solid tumours greatly exceed the leukaemias in numbers. Thus public health risk assessments will understandably attach more importance to the occurrence of solid tumours. However, if the objective is investigative and the aetiology is to be established, then the
occurrence of leukaemias is of paramount importance as an indicator of eventual exposures to radiation beyond the natural background level.

The term leukaemia is a general one used to designate a group of related malignant diseases of the blood. The classification of leukaemias is according to the predominant cell type and the duration from onset to death. The acute forms generally last just a few months while the chronic forms persist over a year and longer. A publication on the RERF findings with regard to the incidence of leukaemias, lymphomas and multiple myeloma for the period 1950 - 1987, reveals that for an adequate evaluation of the consequences of the radiation blast, it is insufficient to lump the leukaemias together as an entity because of the distinct aetiological reactions to radiation of the different types. Further, the practice of excluding malignant lymphomas, CLL (chronic lymphoid leukaemia) and multiple myelomas is inappropriate. The excess relative risk (ERR) determined for the given period was 9.1 for ALL (acute lymphoid leukaemia), 6.2 for CML (chronic myeloid leukaemia) and 3.3 for AML (acute myeloid leukaemia). The frequency of distribution of cases (incidence) was dependent on dose, age, time since exposure and sex.

**Pre-conceptional paternal exposure & childhood leukaemia**

Although the RERF data have undoubtedly contributed substantially to the understanding of radiation leukaemogenesis, the doses involved are much too high to bear any relevance to public health issues. Leukaemogenesis in environmental settings and as a result of medical applications (radiography) involve very low doses of radiation. In recent years some insight into the biological mode of action of low doses has been gained. One of these is the genetic path via pre-conceptional parental exposure.

The question of pre-conceptional medical radiography and cancer risk had been investigated in the USA in a case-control study as early as 1966. The results were, however, regarded as inconclusive even when increased risk for leukaemia was established for certain diagnostic exposures. At that time, the investigators were probably prudent to have left the question open, since such a concept way ahead of contemporary radiobiology would have been dismissed. Accrued epidemiological evidence suggests that paternal preconceptional exposure increases the risk for childhood leukaemia.

The investigations of Martin Gardner on the Seascale cluster of childhood leukaemia, in Britain, led him on the track to the Sellafield nuclear fuel reprocessing plant, where the fathers of the children were employed. These men had received radiation exposures at the plant several months prior to the conception of their children. The postulate on paternal exposures and risks of childhood leukaemia was inevitable by nature of the case-control study. Since the doses involved were very low, this finding triggered off vehement controversy. This finding is now supported by further epidemiological and experimental data.

A case-control study on childhood leukaemia in association with diagnostic X-rays conducted in Shanghai indicated a general association of risk increase and numbers of preconceptional exposures of the fathers. This trend was significant for both ALL and ANLL (Acute non-lymphoid leukaemia). The same author conducted a further case-control study in the United States in which the exposures for both parents were defined according to anatomical regions. This study also indicated that the risk for infant leukaemia was strongly associated with paternal rather than maternal diagnostic X-ray exposures before conception. Significant risk associations were evident particularly for paternal lower abdominal exposures which was strongly correlated with ALL rather than AML. Maternal exposure could alter the risk relationship only if the exposure was the month before conception, although no dose effect could be established.

As controversial as Gardner’s postulate has been, it was not completely novel, since experimental studies on mice produced evidence which suggested that paternal preconceptional irradiation significantly increased the frequency of lung tumours. A more recent study on murine leukaemia employed the same experimental strategy but with a better corollary to the Gardner investigation: paternal irradiation was by plutonium-239 incorporation and offspring were treated with methyl-nitroso-urea (a chemical carcinogen which induces lymphomas and leukaemia in mice). Paternal exposure to plutonium-239 promoted a significant increase in the leukaemia incidence and also shortened the latency period.

**Maternal exposure & Down’s Syndrome in offspring**

The radiosensitivity of cells during the mitotic phase of cell division has been known for a long time, and the induction of numerical chromosomal aberrations has been demonstrated at low levels of radiation exposure for somatic cells. The extreme sensitivity of oocytes during reductional division
In utero and excessive cancer risks. A subsequent American leukaemia, might in fact be induced during embryonal manifestation of the disorder occurs in later postnatal epidemiologists. This is understandable, since other than a unique temporary constellation of the childhood cancers - brought forward the first evidence of a causal link between diagnostic X-ray exposure and excessive cancer risks. A subsequent American study on obstetric X-ray examinations affirmed the OSCC observations. The supposition that extremely low doses of radiation, as from diagnostic X-rays, could significantly raise the cancer risks of infants was adamantly opposed not just because of the startling implication it had towards human radiosensitivities, but probably more so because it came at an inopportune time of zealous nuclear developments - nuclear energy and weapons programmes - in the western industrial world. It has been counter-argued that the RERF data did not yield comparable findings among children. As said earlier, the RERF data suffers a serious drawback not only due to loss of the first five years' mortalities, but also in the gross underrepresentation of the first trimester in utero cohort, partly because of abortions. Nonetheless, the RERF data continues to be regarded as the radiation biologist's yardstick.

Doll and Wakeford acknowledge that in their entirety the OSCC data suggest an increased risk of childhood cancer at a dose of 1 cGy or a risk coefficient of about 6% per Sv, which is in accord with the 5% per Sv suggested by UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiations). These risk estimates are greater than those worked out by the RERF, whose value was 1 to 4.7% per Sv but not significantly different from zero. The doubling dose for childhood leukaemia has been shown to deviate enormously during embryonal development. The first trimester is the most sensitive phase, the second the least sensitive and the sensitivity of the third trimester is somewhere between the other two. The value of the third trimester corresponds approximately to that of the mean value.

It has also been said that differentiation of haemopoietic stem cells does not occur during the first trimester of foetal development, hence the difficulty in understanding the induction of leukaemia during the first trimester as claimed in the studies on medical exposures. In this regard, it should be pointed out that the yolk-sac with blood islands is well developed by the 8th week of pregnancy in humans. It may be expected that the concentration of haemopoietic stem cells in this rudimentary haemopoietic tissue be exceedingly high. Furthermore, postnatal teratogenic and oncogenic events has been shown to occur in a mouse strain even when irradiated at the zygote stage. And in a large experiment at the University of Colorado, USA, 1,680 beagles were irradiated in utero at defined embryonal stages determined by time post-coitus, at days 8, 25 and 55 which would correspond to human

(meiosis) has been demonstrated in mice. The underlying mechanism is thought to involve radiation effects on the meiotic spindle which is responsible for the equal distribution of the chromosomes between the daughter cells. Being particularly radiosensitive at the time of polar body formation, the meiotic spindle in maturing human oocytes seems predilected to the formation of trisomy 21 (Down's Syndrome), as has been documented in a number of studies. Higher incidences of trisomy 21 have been reported for geographical regions of high background radiation such as the coastal areas of Kerala in India and certain areas of China. These earlier studies have been criticised from various standpoints. A careful analysis of an incidental observation made at the Department of Genetics of the Free University of Berlin, Germany, and follow up investigations revealed that the incidence of trisomy in Berlin and other parts of Germany peaked significantly at a time after the Chernobyl accident which corresponded to the full term of human pregnancy. The question of traditional been focused on teratogenic effects. As mentioned afore, the British OSCC - which was the largest case-control study on leukaemia and other childhood cancers - brought forward the first evidence of a causal link between diagnostic X-ray exposure and excessive cancer risks. A subsequent American study on obstetric X-ray examinations affirmed the

In utero exposure and childhood leukaemia

The embryonal effects of radiation exposure has traditionally been focused on teratogenic effects. Consequently, the question of in utero cancer induction in humans fell into the realm of epidemiologists. This is understandable, since manifestation of the disorder occurs in later postnatal life. The concept that childhood cancers, especially leukaemias, might in fact be induced during embryonal development emerged from epidemiological analyses. As mentioned afore, the British OSCC - which is the largest case-control study on leukaemia and other childhood cancers - brought forward the first evidence of a causal link between diagnostic X-ray exposure and excessive cancer risks. A subsequent American study on obstetric X-ray examinations affirmed the

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first, second and third trimester, respectively. The doses ranged from 0 to 0.83 Gy. The cancer mortality for irradiation at all ages was more than ten times that of expectation\textsuperscript{34, 35}.

Apart from medical exposures, \textit{in utero} correlations of childhood cancers have also been reported for background radiations\textsuperscript{36}. More recently, the association of infant leukaemia and fallout from Chernobyl in a contaminated region in Greece was reported\textsuperscript{37}. A check done by the German Childhood Cancer Registry at the University of Mainz confirmed a similar increase in Germany, but the authors argued against any association with radioactive fallout from Chernobyl because of the failure to established a dose-response effects i.e. the higher contaminated areas did not show higher risks\textsuperscript{38}. However, these authors did not consider the possibility that the higher exposures could have led to abortions. Failure to do so was probably because of the generally low doses involved, and certainly because of the currently predominating radiobiological concepts. The principles and mechanisms of biological response are still far from understood.

\textit{In utero exposure and brain defects}

Cellular radiosensitivity (described by \(D_0\), \(D_q\) and \(n\) values) is derived from dose-effect assays, taking clonogenic inactivation as the end-point. A very serious drawback in this assay system, is the prerequisite that the cells to be tested have high plating efficiencies (PE), which is the percentage of clonogens. High plating efficiencies are encountered only in immortalised and/or transformed cells. Diploid/euploid somatic cells have definite replicative potentials \textit{in vitro} and very low PE. Thus the clonogenic assay system can hardly be applied to characterise euploid embryonal cells, with the result that no data on the radiosensitivities of embryonal cells exist.

Embryonal cellular sensitivities change from stage to stage during development. Biological effects and attending health risks of low level radiation exposure is a current issue discussed above in terms of somatic stochastic effects (cancerigenesis). Late deterministic effects on structural and functional brain development have been quantified at low doses using more sophisticated end-points like learning deficiencies, whereby the threshold dose is exceeding low. The RERF has such a follow up on a cohort of individuals who were exposed \textit{in utero}\textsuperscript{39}. Corresponding results have been documented in experimental studies on guinea pigs\textsuperscript{40} and mice\textsuperscript{41}. The development of the cerebral cortex is a complex process involving intricate cell-cell interactions. During this process the cells are extremely sensitive to radiation action. A conservative general consensus of opinion sets the threshold for overt brain damage at 10 eGy, while functional perturbations may go to any extent below this value, depending on the sensitivities of the endpoints. The sensitivity of embryonal brain cells should be appreciated in terms of adult neuronal tissue which is regarded as highly radioresistant.

\textit{In utero exposure and perinatal mortality}

The poignancy of the matter on the hypersensitivity of embryonal cells is exacerbated by reports on perinatal mortality associated with environmental radiation exposures from radioactive fallout. A forerunner to epidemiological investigations of this nature has been Sternglass\textsuperscript{42}, who associated perinatal mortality in the New England states in the USA, with the aerial atom bomb test series in Nevada. Such studies have been regarded by mainstream scientists as heresy, because the calculated doses to the population could account for such an effect, which in conventional terms is thought of as a deterministic effect with mortality as its endpoint! Although a legitimate critique has been that Sternglass did not present dose estimates, it should be noted, that the United States Department of Energy (DOE), under whose jurisdiction the nuclear tests were conducted, kept information on meteorological data and fallout within the country as classified information not available to the public. Only very recently has some of the data been released and is available as a Report from the National Cancer Institute\textsuperscript{43}. The maps indicate that the radioactive clouds went over to the New England states and adjacent areas from where Sternglass reported his findings. Radiobiological experience, albeit with adult animals, has laid down a fundament clearly defining deterministic effects and the estimated LD\textsubscript{50} values. Even the very lowest of such values falls within the high dose range. Since the calculated exposures arising from fallout generally fall within the low dose range, experts have found it impossible to reconcile with the orders of magnitude in dose difference to account for perinatal mortality. A point of negligence has been the total lack of autopsy findings. Present day radiobiology cannot afford to continue to brush aside the observations of Sternglass because similar findings have been recorded in Great Britain\textsuperscript{44} from fallout from weapons testing, in Germany\textsuperscript{45} from the Chernobyl fallout and likewise in several
European countries which suffered contaminations from Chernobyl. The study of Körblein and Küchenhoff in Germany revealed per trend analysis of data on perinatal mortality two significant deviations which coincided with two peaks for environmental radioactive caesium contaminations, as indicated by in measurements on the caesium activity in cow’s milk. A critique that has been levelled at this study is the failure to demonstrate dose-response relationships. A subsequent large-scale study conducted by Scherb et al. compared the cumulative annual proportions of stillbirths in contaminated (Sweden, Poland, Hungary and Greece) with non-contaminated (Portugal, Spain, Ireland, France and the Benelux) European countries. They discovered a significant increase of stillbirths in contaminated countries with effect from 1986 (Chernobyl disaster) which held on through the years included in the study (i.e. up to 1993). Just like the afore mentioned greater oncogenic potential of low doses of ionising radiations, these data suggest the extreme efficacy of low doses of ionising radiation - albeit as radionuclide incorporation - at reducing the chances of foetal survival. It is not known whether there is a threshold for this effect, and thoughts have yet to be made on eventual mechanisms of action as well as on adequate classification (deterministic or stochastic), within the framework of classical radiobiology. As the doses involved are very low, it reflects the extreme sensitivity of embryonal cells. The doctrine of dose-response holds as long as effects observed are based on the same mechanism of action. Since biological responses to low dose exposures are probably based on mechanisms other than direct genotoxic effects, the persistent demand for evidence of a dose-response relationship for scientific validation warrants rethinking.

**Low dose radiation action (LDRA): Adaptive response**

Small doses of radiation have been demonstrated to induce cellular processes which ameliorate damage from a subsequent acutely applied high dose of irradiation. This phenomenon first observed in animal studies has now been scrutinised in an array of in vitro studies on different cell types. Although it has been observed in various cell types, it is not a general phenomenon, since it has not been observed in pre-implantation embryonal cells and spermatocytes. The bulk of the studies have been done on human peripheral lymphocytes, the end-point being chromosomal aberrations. The conditioning doses must be kept within a specified range of 1 to 20 cGy for success. The cellular repair processes which get stimulated go on over 4 to 6 hr post-irradiation. Change in composition of the cell culture medium can alter the adaptive response and treatment of the cells with inhibitors of protein synthesis ablates the response, thus suggesting the involvement of enzymes. Repetition of the conditioning dose does not augment the protective effect. Although much of the studies have been done on human lymphocytes, the lymphocytes of some individuals do not show adaptive response.

The physiological state of cells in the absence of the adaptive response has been termed the low-dose-hypersensitivity and the triggered adaptive response as induced radioresistance. The phenomenon of induced radioresistance has also been analysed in a variety of cell types of non-mammalian origin. Cell survival studies using mammalian cells required the development of an appropriate clonogenic assay system which is able to resolve changes at doses below 1Gy at which most mammalian cell lines approach 100% survival. The precise determination of the number of cells at risk of clonogenic inactivation in a colony forming assay system has been the technical challenge which has been solved by the development of appropriate instrumentation. With the availabilty of this new methodology, two groups - one at the Gray Laboratories in London and the other...
in Vancouver - have demonstrated in a variety of cell lines derived from human tumours, the existence of an inverse dose-effect (or hypersensitivity) estimated at about a factor of 20. Small conditioning doses of radiation induce radioresistance which increases and merges into the shoulder of the conventional survival curve (see Fig. 2).

The underlying mechanism for this induction of radioresistance is thought to be identical with that of the afore mentioned adaptive response. As this phenomenon has also been observed in non-mammalian cells, it has been suggested that it could be a conserved stress response mechanism in evolution. The hypersensitive state could result either from greater numbers of lesions or from deficiency in repair systems. Evidence is mounting that the induced resistance in the adaptive response is due to stepped up repair efficiency. Agents which block repair mechanisms like aminobenzamide cause protraction of the sensitive state. The key aspect with regard to the stochastic effects of low dose exposures is whether such doses more effectively induce oncogenic events. The absence of adaptive response in early embryonal cells and spermatocytes is cause for concern with respect to the aetiology of childhood leukaemias, since epidemiological evidence has underlined the relevance of first trimester in utero as well as paternal exposures to low levels of radiation.

**LDRA: Variation in human radiosensitivity**

Since the formulation of the Target Theory by Timofeeff-Ressovsky et al. in the thirties, this envisionment of radiation action through the inactivation of critical cellular targets by hits had dominated the thinking among the physically oriented radiobiologists into the present day. The cellular target has never been defined, but the discovery of DNA provided the best candidate. Research on the effects of radiation on DNA has therefore continued to be at the core of radiobiology. Ionising radiations cause a whole spectrum of lesions to DNA, none of which is unique since other agents can cause identical damage. However, unique to ionising radiation action is the simultaneous presence of all types of damages. Other agents are more specific in their damaging properties. Depending on the LET (linear energy transfer), the proximity of lesions to one another may also be peculiar of ionising radiations. The major forms of damage to DNA consists of altered bases, missing bases, incorrect bases, bulges in the DNA backbone due to deletions or insertions of a nucleotide, linked pyrimidines, strand breaks, cross-linked strands, DNA-protein cross links and deoxyribose fragments. Each of these lesions can be repaired by DNA repair enzymes. Although all of these lesions are repairable, what determines the fate of a cell is (a) the repair fidelity, (b) the probability of incomplete repair, (c) the probability of misrepair and (d) locus of an error i.e. (b)/(c), if present. The consequences of repair infidelity are mutations and/or cell death (usually loss of reproductive integrity). The problem underlying oncogenic events is misrepair and consequent mutagenesis, followed eventually by additional mutations which facilitate the bypassing of apoptosis and hence cellular transformation.

It is now known that a large number of genes (and their products) are involved in the normal housekeeping of DNA. Defects or absence of the products of these genes can lead to neoplastic development because of new mutations arising from infidelity of DNA replication. Persistence of DNA lesions due to the absence of a repair pathway can escalate errors/misrepair. Many genetic diseases resulting from DNA repair-deficiencies have been studied. The earliest of these has been *Xeroderma pigmentosum* (XP), the cells of which are hypersensitive to UV light because of a deficiency in the excision enzymes required to remove thymidine dimers, a lesion characteristic for the action of UV-B light. *Ataxia telangiectasia* (AT) and *Cockayne's Syndrome* (CS) patients are hypersensitive to ionising radiation. The cells of AT patients have been shown to repair damage from UV light normally but are not able to repair other DNA lesions, especially strand breaks, caused by X-rays. Persons suffering from *Fanconi's Anaemia* (FA) are also hypersensitive to ionising radiations, the reason being that their cells are predisposed to DNA cross-linking. In the case of *Bloom's Syndrome and Fragile X Syndrome* (FXS), cells are subject to a high incidence of spontaneous chromosomal breaks, which *per se* predisposes such persons to be hypersensitive to ionising radiations. The affected persons are highly predisposed to developing cancers. Inheritance of these disorders is generally through autosomal recessive transmission. The molecular characterisations of the deficiencies, excepting perhaps XP, are all in their infancies, with the result that the mechanisms are not understood. Some eight XP genes and genes from AT cells have been cloned and their activities are being analysed *in vitro*. The sensitivities of recessive carriers of traits in a population (heterozygotes) to radiation, and their frequencies are not definitely known.
Health disorders attributable to DNA repair deficiencies can be considered to be a special and better studied example - by virtue of the preoccupation of radiobiologists with DNA as the candidate target for radiation action - of a more generalised phenomenon of ecogenetic traits. Genetic factors influence individual responses to environmental cancerogenic agents. The study of gene polymorphisms as the basis for hypersensitivities and predispositions to neoplastic developments has began and an expanding list must be expected in the future. Given the number of proteins involved in DNA surveillance and repair, one might expect that a significant fraction of the general population may carry mutations which predispose them to higher cancer risks, because of deficiencies in restoring cellular damage. This aspect has as yet to be given due consideration in the setting of exposure limits in radiation protection.

**LDRA: Cytoplasmic response and epigenetics**

Radiation effects on cell membranes has been a subject of interest in the early years, but faded into disregard because of the predominating developments in the area of cell nuclear and DNA effects of radiations. Furthermore, since the radiation doses required for inducing functional changes in cell membranes were generally by far higher than what was required in the DNA studies, it fortified the notion that cytoplasmic effects were irrelevant. The current revival of attention towards the cytoplasm and even towards the extracellular matrix (ECM), is due to the improved general understanding on the reactions of biological systems to toxic stresses. It is now clear that cellular reactions to ionising radiation - or any other toxic agent - depend on the degree of organisation i.e. whether the cells are isolated, or in a dense population of interacting cells. Cell-cell interactions are of paramount importance and depends on the functional integrity of the ECM, the structural constituent of the cellular microenvironment. Oncogenesis is often emphasised as being a multistep process. It is now recognised that the process is subject to microenvironmental control i.e. the interactions of the affected cell with neighbouring cells and with the ECM, which comprises of a supporting macromolecular polymer-meshwork to which the cells anchor by aid of attachment proteins like fibronectin. The spaces in between are filled by molecules essential for the maintenance of metabolic and regulatory processes. In normal function, gene expression is not an autonomous event but a concerted effort between the genome, the cytoplasm (mediated via cytoskeletal elements and membrane integrins) and the cellular microenvironnement.

Radiation induced stochastic effects must first bypass apoptosis before oncogenesis can occur, since apoptosis is a control mechanism leading to the elimination of such affected cells. Once this has been circumvented, the growth regulatory (suppressive) mechanisms of the cellular microenvironment must be undermined. This may be achieved through adequate genomic alterations i.e. the unscheduled up/down regulation of gene activities. Theoretically, any dose of radiation above zero level is potentially cancerogenic. It is only a question of probabilities, the probability being low at low doses. Epidemiological studies have demonstrated that low levels of exposure provoke risks comparable to high level exposure. Very recent experiments in cell cultures are beginning to provide the missing biological rationale. The use of alpha particle microbeams has been an important tool for delivering discrete low doses (number of particle traversals) to defined parts of a cell. Thus, it has now been shown that irradiation of the cytoplasm provokes DNA mutations of a profile corresponding to the naturally occurring background type, while irradiation of the nucleus produced gross multiloci mutations, as is generally known for radiation action. Viability of the latter is low, while the former is characterised by high viability and, therefore, higher cancer potential. In general, at high dose levels, when irradiation is not restricted to a part of the cell, as dose increases the amount of cell killing (loss of reproductive integrity) increases exponentially. On the other hand, at low doses, cell killing is negligible, although death through apoptosis may compensate to some extent. The chances for viable misrepairs are distinctly improved.

It is not as yet established beyond doubt whether suprasensitive sites, predestined to be targets for radiation action, are present in the genome. Some investigations have suggested that the sites of attachment of chromatin to the nuclear envelope and to the nuclear scaffold are particularly sensitive to radiation action. Whether these purported sites trigger oncogenic events is not known. Otherwise, they could have been regarded as candidates for low dose radiation action. These sites of chromatin attachment are linked to the cytoskeleton of the cell through attachment of cytoplasmic microfilaments which also link on to the ECM via membrane-bound proteins called integrins. In the above-mentioned paper on the mutagenicity of alpha particle traversal...
through the cytoplasm, the authors attempted to elucidate the probable mechanism of action, which they suspected to be free radicals. This was tested by ablation through dimethyl sulfoxide (DMSO), which failed when the nucleus was irradiated. Calculations for the free radical half-time and the distance of the nucleus from the cytoplasmic site of irradiation did not support the free radical concept. The eventual involvement of cytoskeletal elements in transducing a remote effect was not considered by the authors, although it is a likely possibility.

As said, the expression of mutagenicity as well as oncogenicity is influenced by the cellular microenvironment. Its depth of implication is best exemplified by two complementary phenomena which have been called the Bystander Effect and the Good Samaritan Effect, the latter being effectively the opposite of the former. Both effects essentially underline the ability of cells to convey negative (bystander) or positive (good samaritan) influences to neighbouring cells. The bystander effect has been observed at low dose radiation exposures with alpha particles, since the fluence in terms of the number of particle traversals can be regulated. Little and associates\(^5^5\) demonstrated that cytogenetic effects caused by exposure to alpha particles in the dose range of 0.03 to 0.25 cGy was propagated from the target cells to bystanders. It was noted that 30 to 50\% of the cells in the total population contained sister chromatid exchanges (SCE), whereas less than 1\% of the cells were traversed by alpha particles. The effect could be abrogated by the application of Lindane (gamma isomer of hexachlorocyclohexane), a gap junction inhibitor\(^5^6\). And as the bystander effect is not observed in non-confluent cell cultures, the significance of cell-cell interactions is underlined. The relevance of the bystander effect to neoplastic transformation has also been shown in an in vitro mutagenesis system where C3H10T1/2 (immortalised) cells were irradiated with beta particles. Cells neighbouring irradiated cells transformed at a frequency which was ten times greater than those further away where cell-cell contacts to the irradiated cells could be ruled out\(^5^5,5^8\). Thus epigenetic effects in carcinogenesis may find their explanations in cell-cell interactions and other cellular microenvironmental influences which include biologically active humoral factors like eicosanoids (prostaglandins and leukotrienes), cytokines and chemokines.

**LDRA: Induced genomic instability**

Genomic instability has since a long time been recognised as a property of cancerous cells, and systematisation of the cytogenetic alterations that certain types of cancers go through, either as a result of treatment or spontaneously, has been exploited for prognostic purposes. Although this phenomenon has recently caught the attention of radiation biologists and oncologists alike, its manifestation has been recorded in some older studies (genealogy of the progeny of irradiated cells) without being fully appreciated. The credit for having brought this important fundamental phenomenon into the limelight of current research interests goes to Kadhim et al for their two landmark papers\(^5^9,6^0\). What they observed is now the basis for defining genomic instability, which is the increase in de novo acquired genomic alterations in the progeny of affected cells, which may be manifested as mutations, gene amplifications or chromosomal aberrations. A step up in chromosomal aberrations have been observed in the progeny of irradiated cells even 30 to 40 cell generations later. Genetically linked disorders observed in humans hypersensitive to ionising radiations are known to suffer deficiencies in their DNA repair systems (vide supra). They are now known to also manifest genomic instabilities. The type of chromosomal aberrations observed in the progeny of irradiated cells have been said to be similar to the aberrations observed in cancer cells in the course of progression, a development which has been attributed to genomic instability. This only affirms a fundamental principle of radiation carcinogenesis which is to step up naturally occurring stochastic events. The studies of Kadhim et al\(^6^1\) referred to above showed that the genomic destabilisation provoked by alpha irradiation of progenitor cells, was independent of dose i.e. lower doses had the same potential or were just as efficacious as higher doses. In a recent paper they showed that the traversal of a single alpha particle was sufficient to trigger genomic instability. This finding demonstrates the higher oncogenic efficiency of low doses. Epigenetic mechanisms are certainly important.

Genomic instability now offers a basis for the understanding of radiation induced oncogenesis and the aetiology of several genetically linked disorders in humans. A poorly understood aspect in the radiation aetiology of leukaemias and other cancers has been the latency periods. Focusing on leukaemias, either stem cells or progenitors of the respective cell
compartment must be assumed to become transformed. If the pluripotent stem cell is involved, it is a matter of stochastics as to whether a stem cell receives the appropriate genomic impulses required for transformation. Should that happen, it is again a matter probabilities as to when the affected stem cell might be required to devide. There is obviously no limit as to how many mitotic events may be required for a destabilising event to reach a critical stage, such that the cell and further progeny acquire the required property of liberation from local regulatory factors, to overtly manifest their transformed nature in way of uncontrolled proliferation (clinically then recognisable as pre-leukaemic). It is also conceivable that cells of the microenvironment (stromal elements) acquire a radiation injury which does not get eliminated, and becomes processed in such a way as to endow a microenvironmental unit with the property of giving destabilising impulses to haemopoietic precursors during differentiation. The initial damage to the microenvironmental cells could require any extent of time to acquire the oncogenic inductive potential. The few studies which give direct hints in support of what has been postulated here as conceivable mechanisms for explaining latency have been done with alpha particles. It is now an open field for radiobiologists to investigate with reference to radiations of different qualities (low and high LET).

LDRA: Combined radiation effects

The biological effects of ionising radiations can be modified in the presence of other agents like chemicals, drugs, bioactive substances and other physical agents (heat, incorporated fibres, particulates and other categories of the electromagnetic spectrum - UV light, microwaves, radiowaves, ELF). Knowledge on combined radiation effects is restricted to experience which has accumulated in the field of radiotherapy. This has mainly involved the application of certain drugs which offer radioprotection/radiosensitisation, in efforts to improve the efficacy of radiotherapy. The application of hyperthermia has also served the same purpose. There is an enormous gap in knowledge when it comes to environmentally oriented problems of low level radiation exposure, especially from internal emitters, in combination with other toxic agents present in the environment. These in dependence on individual susceptibility have never been investigated because of the technical difficulties which such studies pose.

Concluding remarks

The spread in human radiosensitivity is not known. Besides conspicuous genetically linked disorders leading to hypersensitivity in homozygotes, a fraction of any population must also be expected to be heterozygous bearers of those recessive traits. The sensitivities of these individuals and those who bear variations in enzyme systems responsible for dealing with cellular oxidative stress are not known. The genetic traits provide the basis upon which the other afore-mentioned mechanisms act. Environmental exposures will always be in combination with other factors and the challenge of the future, in making risk assessments, will be to give due consideration to the various factors which can influence oncogenetic events.

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