Radiation carcinogenesis in experimental animals

J. J. Broerse[a, b], D. W. van Bekkum[a] and C. Zurcher[c]

[a] *Radiobiological Institute TNO, P.O. Box 5815, NL-2280 HV Rijswijk,* [b] *Dept of Clinical Oncology, University of Leiden, P.O. Box 9600, NL-2300 RC Leiden, and* [c] *Institute of Experimental Gerontology TNO, P.O. Box 5815, NL-2280 HV Rijswijk (The Netherlands)*

Summary. Exposure of man to relatively high doses of ionizing radiation is generally restricted to accidental situations, with very limited knowledge about the actual doses received. Animal experiments can be performed under standardized and controlled conditions and can provide information on the dose-response relationships for radiation carcinogenesis.

The risk of inducing neoplastic late effects after total-body irradiation with relatively high doses has been demonstrated for larger animals, such as monkeys and dogs. The bone marrow, the mammary glands and the lungs are among the tissues with the highest susceptibility for radiation carcinogenesis. Experimental results on tumour induction in rodents are summarized with emphasis on the effectiveness in dependence on radiation quality and fractionation or dose rate.

Key words. Tumour induction; dose-response relations; relative biological effectiveness; fractionation; dose rate.

Introduction

Tumour induction is generally considered to be the most important late effect of exposure to ionizing radiation. The carcinogenic effects can be modified when the time course of the irradiation is changed by fractionation or protraction. The radiation quality, quantitatively expressed as linear energy transfer (LET) influences the detrimental effects. Radiations of differing LET have a different relative biological effectiveness (RBE) for many biological endpoints including tumour induction.

For doses below 0.3 Gy of low-LET radiation, such as gamma rays, epidemiological data have not provided reliable evidence for the carcinogenic effect of radiation in man. Information on the effects of high-LET radiation, such as neutrons, in human populations is completely lacking. Studies in experimental animals have therefore been performed with the objectives to assess the nature of dose-response relationships, to determine the RBE of radiations of different quality and to investigate the influence of fractionation or protraction of the dose on tumour development.

Exposure of patients to high-dose total-body irradiation followed by bone marrow transplantation, has shown to be increasingly successful for the treatment of malignancies such as leukaemia as well as for aplastic anaemia. Partial-body irradiation has been effective for treating Hodgkin's disease. For long-term surviving patients it will be important to assess the risk of inducing neoplastic late effects. Studies on tumour induction in monkeys and dogs after whole-body irradiation with X rays or neutrons have provided data relevant to this issue.

Cancer may be induced by radiation in nearly all tissues of the human body. However, it has been shown that tissues and organs can vary considerably in their sensitivity to the induction of cancer by radiation. The reassessment of the radiation dosimetry for the survivors after the atomic bomb explosions at Hiroshima and Nagasaki[23] have resulted in increased risk factors for mortality due to radiation-induced tumours in different organs[22, 26] in comparison with earlier recommendations[14]. A number of tissues with high sensitivity for cancer are summarized in table 1. The mortality risk factors, derived from an absolute risk model, are representative for exposure at high doses. It should be noted that the spontaneous incidence of mammary cancer in the Western countries is high with respect to that in Japan. For stomach cancer in males a higher spontaneous incidence is observed in Japan than in Western countries. The total mortality risk factor for radiation-induced tumours would be equal to $4.2 \cdot 10^{-2}$ Sv^{-1} (Sievert) as an average for men and women, according to the absolute risk model and $6.9 \cdot 10^{-2}$ Sv^{-1} according to the relative risk model. The introduction of a reduction factor varying between 1 and 3 to convert the risk at high doses to that at low doses and low dose rates is still under discussion. Studies on tumour induction in experimental animals can provide pertinent information concerning the extrapolation procedures.

The bone marrow, the mammary glands and the lung are among the tissues with the highest susceptibility for radiation carcinogenesis. Experimental results on tumour induction in mice and rats are summarized with emphasis on the effectiveness in dependence on radiation quality and fractionation or dose rate.

Table 1. Sensitivity of various tissues to oncogenic influence and related mortality risk factors

Tissue or organ	Spontaneous incidence of cancer	Relative sensitivity for radiation carcinogenesis	Mortality risk factor (Sv^{-1})
Female breast	+++	++	$4.3 \cdot 10^{-3}$
Lung	+++	++	$5.9 \cdot 10^{-3}$
Stomach	+	+++	$8.6 \cdot 10^{-3}$
Large intestine	++	+	$2.9 \cdot 10^{-3}$
Bone marrow	+	+++	$8.4 \cdot 10^{-3}$

Tumour induction after high dose irradiation

Patients suffering from malignancies of the lympho-haemopoietic system have been conditioned for bone marrow transplantation by administration of cytotoxic drugs or relatively high doses of total body irradiation. In view of the concern about the induction of secondary malignancies in human bone marrow graft recipients, studies in animals are of value. Experiments in nonhuman primates are relevant in this respect since the response to radiation of these species does not seem to be significantly different from that of the human[4].

Deeg et al.[11] exposed dogs to ^{60}Co gamma radiation with a single dose of 6.1–11.4 Gy or fractionated irradiations with total doses of 7.6–21.3 Gy. The exposed dogs had an estimated risk of developing a malignancy five-fold higher than in control dogs.

Partial body irradiations of beagle dogs were performed by Bradley et al.[3] with the aim of investigating early and late effects on canine brain, spinal cord and lung when clinical fractionation schedules are applied. The doses used were 10–26 Gy of d (35) + Be neutrons with a mean energy of 15 MeV and 35–79 Gy of ^{60}Co gamma-rays. From the group of 46 dogs irradiated with collimated neutron beams, seven dogs developed neoplasms within the radiation field. For the group of neutron irradiated dogs at risk, the incidence of neoplasia was 15% with a zero incidence in the controls.

Accidental exposure of man to ionizing radiation will generally result in a rather inhomogeneous irradiation. Experiments under standardized and controlled conditions can be performed with biological systems. Because of both its practical and fundamental interest, the response of Rhesus monkeys after exposure to relatively high doses and the protective effect of autologous bone marrow transplantation have been investigated[4]. For these investigations special provisions have to be made to achieve an irradiation of these relatively large animals with a dose distribution which is as homogeneous as possible. For the non-grafted monkeys, the LD_{50} values for X- and neutron-irradiated monkeys are 5 and 2.6 Gy, respectively, resulting in an RBE of approximately 2 for the occurrence of the haemopoietic syndrome. Protection by autologous bone marrow transplantation was demonstrated for doses up to 8 Gy of X rays and 4.4 Gy of fission neutrons. For higher doses the bone marrow grafting was no longer effective and the majority of animals died within seven days with severe damage to the small and large intestine.

The risks of total body irradiation with doses of 6–8 Gy are investigated by keeping the long-term surviving monkeys from the above described experiment under continuous observation. Rhesus monkeys of a comparable age distribution were maintained under identical conditions of housing and nutrition to serve as a control group. The animals were inspected frequently. In some cases the animals were killed when moribund; as soon as possible after the death of an animal, a complete necropsy was performed. The average absorbed doses in the animals were equal to 6.7 Gy and 3.4 Gy, respectively, for the monkeys irradiated with X rays and fission neutrons. The data on longevity and tumour incidence in the irradiated monkeys and in the control group are summarized in figures 1 and 2, respectively.

The latency periods for neoplastic diseases varied between 7.5 and 15.5 years for X-irradiated animals and between 4 and 18 years after neutron irradiation. In the X-irradiated group 9 out of 20 monkeys, and in the neu-

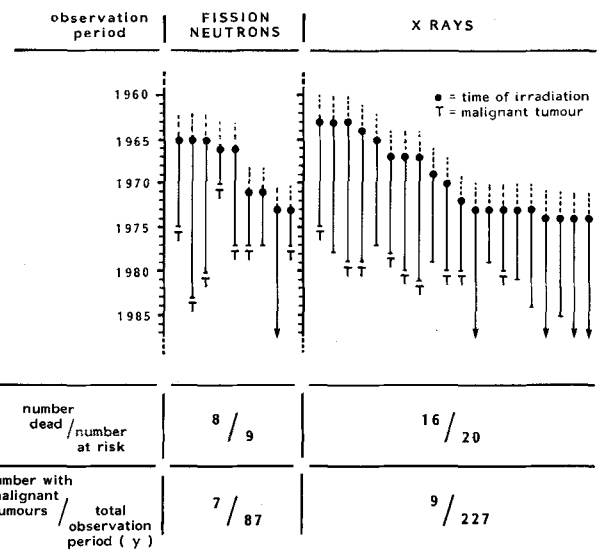

Figure 1. Tumour incidence and post-irradiation observation periods for long-term surviving Rhesus monkeys after whole-body irradiation and autologous bone marrow transplantation. The dashed portion of the lines indicate the approximate age of monkeys before entering the colony. Lines ending in cross bars signify death and arrow heads indicate that the monkeys are still alive.

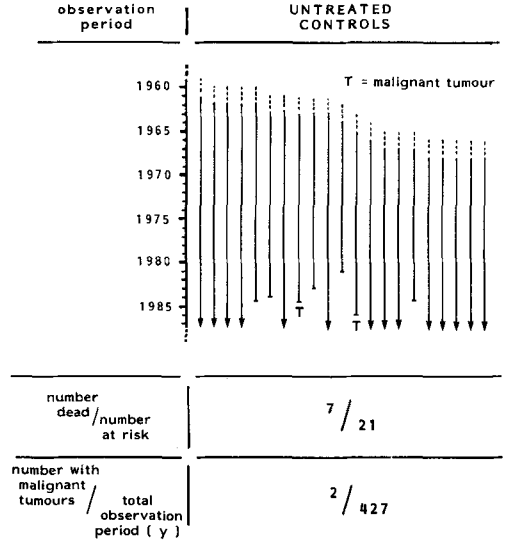

Figure 2. Tumour incidence and observation periods in untreated Rhesus monkeys which served as an age-matched control group for the experiment on radiation carcinogenesis.

Table 2. Neoplasms observed in X-irradiated Rhesus monkeys

Sex	Post-irrad. interval(y)	Average whole body dose (Gy)	Neoplasms Malignant	Benign
♂	10	3.7	–	Adenoma, parathyroid
♂	12	7.1	Papillary adenoCA, kidney	
♀	14	7.1	Epitheloid Schwannoma, knee	–
♀	14.5	7.5	–	Fibroleiomyoma, uterus
♀	15.5	7.5	Follicular CA, thyroid	Fibroma, skin Neurofibroma, skin
	7.5	7.9	Papillary adenoCA, kidney	–
♂	8	7.9	Papillary adenoCA, kidney	–
♂	9.5	7.9	Mucinous adenoCA, colon Papillary adenoCA, kidney	–
♂	11	8.0	Osteosarcoma, calvarium	Adenoma, pituitary
♂	12	8.0	Glomus tumour, subcutis osteosarcoma, maxilla	Adenoma, kidney
♂	15	8.0	Papillary adenoCA, kidney Follicular CA, thyroid	Papillary cystadenoma, kidney Neurofibroma, skin Adenoma, pituitary Lipoma, peritoneum

Table 3. Neoplasms observed in Rhesus monkeys after irradiation with fission neutrons

Sex	Post-irrad. interval (y)	Average whole body dose (Gy)	Neoplasms Malignant	Benign
♂	10	2.3	Glomus tumour, pelvis	Islet cell adenoma
♂	15	2.6	Glomus tumour, scrotum Cortical carcinoma, kidney	Hemangioma, subcutis Neurofibromas, adrenal pheochromocytoma Islet cell adenomas
♂	11	3.5	Osteosarcoma, calvarium Papillary cystadenoCA, kidney	
♂	18	3.5	Synovial sarcoma, shoulder	–
♂	4	3.8	Glomus tumour, subcutis Osteosarcoma, humerus	–
♂	6	4.1	Astrocytoma, cerebrum	Fibroma, skin
♀	4	4.4	Glioblastoma, cerebrum	–

tron group 7 out of 9 animals died with malignant tumours. The results of the pathological findings for the two radiation modalities are summarized in tables 2 and 3. The incidence of intracranial tumours is in accordance with the results of Yochmowitz et al.[35], who performed a lifetime study in monkeys after single total body exposure to mono-energetic protons with doses between 1 and 8 Gy. The induction of malignant glomus tumours is of interest, since this tumour type is rarely observed in man. In the group of 21 untreated control monkeys, two animals died at the age of 23 years with uterine cervical cancer and gastric cancer, respectively. Although the total number of animals included in our study is rather limited, it is noteworthy that the mortality with cancer (two out of seven) in the group of unirradiated monkeys, resembles the situation in the aging human population. As indicated in the figures, the results can be analysed in terms of the number of animals developing tumours per group as a function of the total observation period for the entire group and the average absorbed dose. Thus in the neutron-irradiated group, seven monkeys developed malignant tumours in a total observation period of 87 monkey-years. For the X-irradiated group this number is equal to 9 per 227 monkey-years and for the control group, 2 per 427 monkey-years. In this way risk factors for tumour induction of $9 \times 227^{-1} \times 6.7^{-1} = 59 \times 10^{-4}$ year^{-1} Gy^{-1} for X rays and $7 \times 87^{-1} \times 3.4^{-1} = 237 \times 10^{-4}$ year^{-1} Gy^{-1} for fission neutrons and an RBE of 4 can be derived. It should be realized that these risk factors are calculated on the assumption of a linear dependency of the dose-effect relationship; that they are derived from tumour incidence data obtained at relatively high doses and that they pertain to risk factors per monkey-year. Furthermore, this approach does not take into account the time-dependence of the tumour appearance. If one applies the concept of cumulative tumour rate, the cumulative hazards are 0.64 and 0.18 for the neutron and X-ray experiment at 10 years past irradiation. On the basis of such a calculation the RBE for tumour induction in Rhesus monkeys would be equal to $0.64 \times 3.4^{-1}/(0.18 \times 6.7^{-1}) = 7$. It is clear that the two different approaches can produce some ambiguities in the assessment of risks resulting from different types of radiation.

Analysis of tumour incidence data

A common approach in the analysis of tumour induction studies in animals is to score the fraction of animals that develop a tumour in course of time. The tumour incidence results should be analysed, however, by actuarial

methods correcting for loss of animals from the experiment due to intercurrent disease or other reasons. The fraction of animals surviving without evidence of tumour can recursively be calculated as:

$$P(t_k) = P(t_{k-1}) \frac{n_k - \delta_k}{n_k}$$

For the calculation of this survivor function successive observation periods, t_k, are considered, $P(t_0) = 1$, n_k is the number of animals at risk during the time interval (t_{k-1}, t_k) and δ_k is the number of animals showing a first tumour in this time interval.

The probability curves for survival without tumours, derived by the Kaplan and Meier[16] product limit estimate, inevitably show a step-wise dependence as a function of time. The stochastic effect of carcinogenesis can be described by hazard functions which result in curves with a common shape for groups irradiated with increasing dose. Such descriptions have been performed by applying non-parametric models, e.g. the proportional hazards model[17] or analytical models, e.g. the Weibull distribution[15].

As an alternative to the proportional hazards model, Chmelevsky et al.[8] utilized a joint maximum likelihood analysis for the induction of pulmonary neoplasms in rats after radon inhalation or irradiation with fission neutrons. The prevalence functions of lung carcinomas in Sprague Dawley rats as analysed with an accelerated failure time model (AT) or a shifted time model (ST) are in general agreement, as can be seen from figure 3. The use of the same model for the two radiation modalities makes it possible to determine the equivalence between the working level month (WLM) value and the neutron-absorbed dose.

Data on radiation-induced mammary cancer in different rat strains after irradiation with X rays, gamma rays and mono-energetic neutrons have been analysed by an analytical hazard model[5]. The most important quantities in this analysis are the hazard function, h (t), and the cumulative hazard function, H (t), which are correlated in the following way with the survivor function:

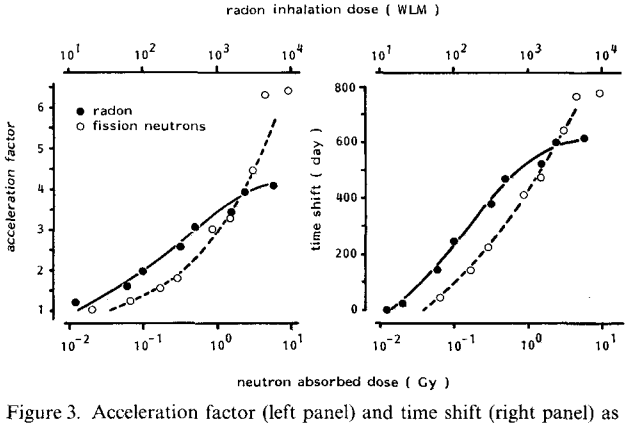

Figure 3. Acceleration factor (left panel) and time shift (right panel) as a function of either radon-inhalation exposure or neutron absorbed dose[8].

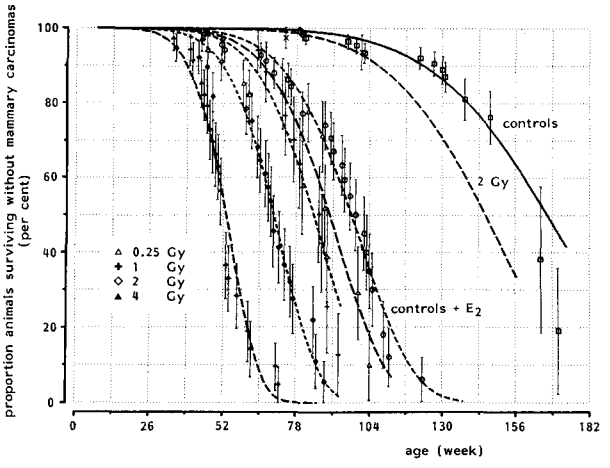

Figure 4. Probability of surviving without evidence of carcinomas in WAG/Rij rats after X-irradiation and administration of the hormone E_2 one week prior to irradiation. The two right-hand curves refer to response of the animals without hormone administration[7].

$$P(t) = \exp\{-\int_0^t h(u)\,du\} = \exp\{-H(t)\}$$

The survival curves can consecutively be analysed with a Weibull distribution:

$$P(t) = \exp\{-(t-\gamma)^{\beta}(1/\alpha(D))^{\beta}\}$$

where α is the time scale parameter, β the shape parameter and γ the location parameter, which indicates the time prior to which tumours have not been observed. As an example of this approach, the results on induction of mammary carcinoma in WAG/Rij rats after X-irradiation in combination with hormone administration are shown in figure 4.

The relative excess hazard can be defined independently of the time function and describes the net effect in the hazard of an irradiated cohort relative to the hazard of the control cohort as:

$$\eta(D) = \frac{H(t, D) - H(t, 0)}{H(t, 0)} = \left\{\frac{\alpha(0)}{\alpha(D)}\right\}^{\beta} - 1$$

In order to obtain complete information on in vivo carcinogenesis results it will be essential to keep the animals under observation during their full life span and to include adequate control groups of comparable age distribution. The tumour incidence data should be based on histopathological examination. In most analyses the moment of observation of a palpable tumour is taken as an endpoint. This implies that for microscopic tumours found upon obduction a slightly different mathematical approach should be followed than for grossly visible tumours[6]. It will be of scientific and practical interest to

analyse the same set of tumour induction data by different mathematical models.

Nature of dose response relationships

When all appropriate corrections have been applied, the dose-response relationships for radiation-induced cancer can, in their most general way, be described as:

$$I(D) = (a_0 + a_1 D + a_2 D^2) \cdot S(D)$$

where I (D) is the incidence after exposure to an absorbed dose D, a_0 is the spontaneous incidence, a_1 and a_2 are coefficients for the linear and dose-squared terms of cancer initiation and S (D) is the probability of survival of transformed cells. The quotient of the induction constants (a_1/a_2) varies with the radiation quality and the specific biological effect. For tumour induction in most tissues, the influence of the cell kill factor will not be very important in the range of doses up to 1–2 Gy of low-LET radiation (or their high-LET equivalent). It has been pointed out[23] that the linear-quadratic model of cancer initiation is a simplistic concept and not a pathogenic theory. The great complexity and the interaction of various phenomena at the cellular and tissue level preclude its applicability for cancer induction in all tissues and under all circumstances. An extensive review of dose-response relationships in experimental systems has recently been given by UNSCEAR[31]. The induction of skin cancer in rats and mice by local low-LET irradiation requires high doses and apparent or real thresholds can not be excluded, nor can they be established. For the induction of bone sarcoma after incorporation of Ra-226 and Sr-90 in dogs and mice, a practical threshold is indicated.

Studies on mammary tumorigenesis performed at TNO in three rat strains, notably Sprague-Dawley, WAG/Rij and BN/BiRij have shown that only in WAG/Rij rats an appreciable number of carcinomas was induced by irradiation[36]. Analysis of the data by hazard functions has resulted in linear dose-response curves for fibroadenomas in Sprague Dawley rats and for fibroadenomas and carcinomas (fig. 5) in WAG/Rij rats after irradiation with 0.5 MeV neutrons and X rays. It should be recognized, however, that the dose-response curves for the X-irradiations do not comprise experimental points for dose levels below 0.2 Gy.

In order to investigate the risk of repeated exposure to very small doses of radiation as encountered in screening programs by mammography, the induction of mammary tumours has been studied in the WAG/Rij strain after fractionated irradiations with relatively low doses of gamma rays[1]. The effects of fractionated irradiation with Cs-137 gamma rays of 120 fractions of 2.5 mGy and 10 mGy (interval 12 h) are compared with those of an acute exposure with doses of 0.3 Gy and 1.2 Gy. In general, the animals are introduced to the exposure regimen at the age of 8 weeks. In view of a possible dependence

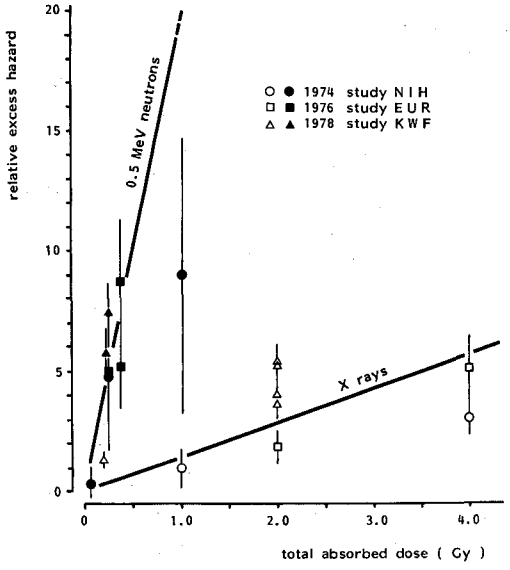

Figure 5. Relative excess hazard as a function of total absorbed dose for the induction of carcinomas in WAG/Rij rats after irradiation with X-rays and 0.5 MeV neutrons as obtained in three consecutive experiments.

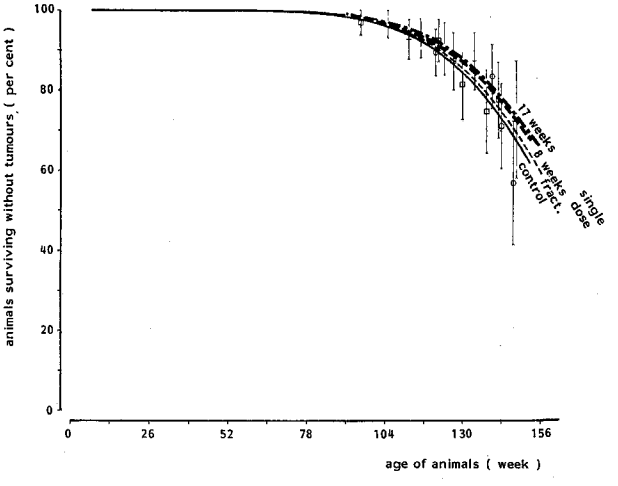

Figure 6. Probability of surviving without evidence of mammary carcinomas in WAG/Rij rats after single-dose irradiation at 8 and 17 weeks of age and after fractionated irradiation with 300 mGy gamma rays in comparison with controls.

of susceptibility on age and developmental stage of the mammary glands, two additional groups were irradiated with single doses at the age, reached at the end of the fractionated irradiation, notably 17 weeks. The experiments were performed with normal animals and animals in which estradiol-17β (E_2) pellets were implanted subcutaneously at the age of 6 weeks. Earlier experiments have demonstrated the promoting action of this natural hormone[7].

The time dependence for survival of rats without evidence of either benign or malignant tumours has been analysed. The occurrence of mammary carcinomas in animals without hormone treatment after irradiation with 0.3 Gy or 1.2 Gy is shown in figures 6 and 7. These

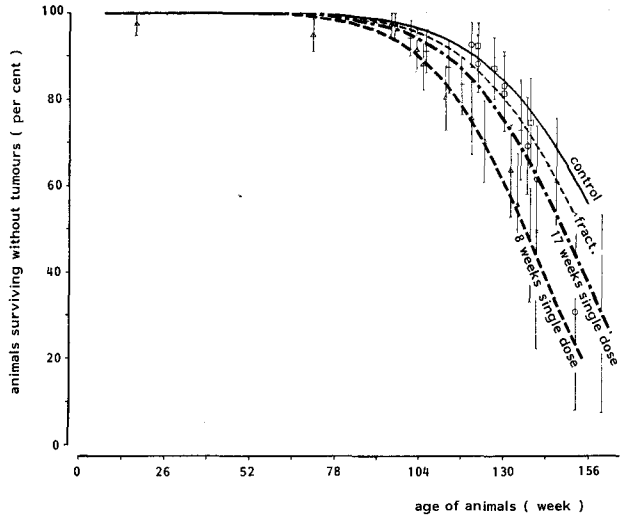

Figure 7. Probability of surviving without evidence of mammary carcinomas in WAG/Rij rats after single-dose irradiation at 8 and 17 weeks of age and after fractionated irradiation with 1200 mGy gamma-rays in comparison with controls.

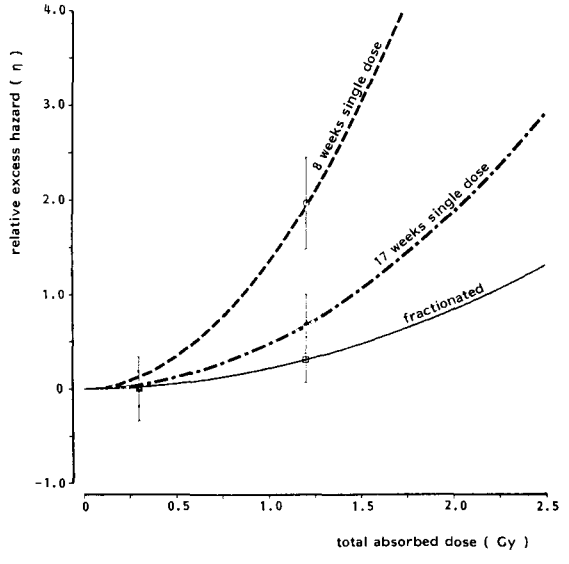

Figure 8. Relative excess hazard as a function of the total absorbed dose for the induction of mammary carcinomas in WAG/Rij rats after gamma-irradiation.

results form the basis for the calculation of the relative excess hazard as a function of the total absorbed dose (fig. 8). The values for the excess hazards for carcinomas as well as for fibroadenomas after irradiation with 0.3 Gy total dose are too small to be of statistical significance. For the 1.2 Gy exposure, the results are comparable for the carcinomas and the fibroadenomas, in that the single-dose irradiation at 17 weeks of age is less tumourigenic than the single dose at 8 weeks of age. For both tumour types, fractionated irradiation results in significantly fewer tumours than after the single exposures. On the basis of the available data, the values for the relative excess hazard for induction of mammary tumours can mathematically be described as:

$$\eta(D) = a_1 D + a_2 D^2$$

The resulting values for the linear (a_1) and quadratic (a_2) components are summarized in table 4. It can be seen from the table that quadratic dose-response curves without a significant linear component are derived for the induction of carcinomas. The susceptibility for tumour induction is considerably reduced when the irradiation is performed at an older age.

In a restricted number of studies, tumour induction has been studied in the same tissue with radiations of different quality, notably X or gamma rays (low-LET) and neutrons or α-particles (high-LET). The nature of the initial parts of dose response curves for carcinogenesis in various tissues with respect to the presence of a linear and/or quadratic coefficient are summarised in table 5. In the intermediate and low-dose region, life shortening is essentially due to a higher tumour mortality. This does not imply that dose-response curves for both endpoints are directly comparable, because, even for the same type of radiation, the mean latency periods of some tumours vary with age at time of exposure and physical factors, such as, dose or dose rate. A separate analysis of ovarian tumour induction from a life shortening experiment in mice [10] substantiated the presence of a threshold dose for X rays. The results of two other experiments on life shortening in mice are included in table 5.

For four experimental endpoints, notably mammary carcinoma in rats [1], lung adenocarcinoma in mice [9], myeloid

Table 4. Linear and quadratic components with standard deviations of the dose-effect relationship for induction of mammary tumours in WAG/Rij rats

Type of tumour Treatment		Carcinoma gamma	gamma + E_2	Fibroadenoma gamma	gamma + E_2
Single-dose irradiation at	a_1 (Gy^{-1})	0	0	0	4.8 ± 0.6
8 weeks of age	a_2 (Gy^{-2})	1.3 ± 0.3	8.1 ± 3.5	2.0 ± 0.5	0
Single-dose irradiation at	a_1 (Gy^{-1})	0	0	0	2.7 ± 0.6
17 weeks of age	a_2 (Gy^{-2})	0.4 ± 0.3	1.6 ± 0.8	1.0 ± 0.5	0
Fractionated irradiation with	a_1 (Gy^{-1})	0	0	0	1.1 ± 0.3
120 fractions	a_2 (Gy^{-2})	0.2 ± 0.1	0.3 ± 0.4	1.2 ± 0.1	0

Table 5. Characteristics of the initial part of dose-response curves for carcinogenesis in various tissues after exposure to radiations of different quality

	Low-LET		High-LET	
	Linear	Quadratic	Linear	Quadratic
Lung adenocarcinoma in mice				
Ullrich[28]	+	+	+	–
Coggle[9]	–	+	+	–
Lung carcinoma in rats				
Chmelevsky et al.[8]			+	–
Myeloid leukaemia in mice				
Mole[21]	–	+	+	–
Ullrich and Preston[30]	+	–	+	–
Mammary carcinoma in rats				
Broerse et al.[5]	+	–	+	–
Van Bekkum et al.[1]	–	+		
Life shortening in mice				
Maisin et al.[20]	–	+	+	+
Thomson et al.[27]	+	–	+	–

leukaemia in mice[21], and life shortening in mice[20] approximately pure quadratic dose-response relationships are reported for low-LET radiation. The apparent absence of a linear term in the dose-response equation for tumour induction after X-irradiation is difficult to explain in the light of microdosimetric data. Apparently, repair processes at the (sub)cellular level and systemic factors can modify the primary process of cancer initiation.

Relative biological effectiveness of fast neutrons

Contrary to directly ionizing radiation, such as α-particles, exponential depth-dose distributions are observed for neutrons which deposit their energy through recoil protons, α-particles and heavy ions resulting from the interactions with tissue. In consequence, fast neutrons can be considered as a convenient radiation modality to irradiate a relatively large biological object with high-LET radiation. The highest RBE values are observed for neutrons with energies of 0.43 to 1 MeV as produced by the p + T reaction or by the fission process.

For radiation protection applications, the main emphasis is on the assessment of the RBE at low dose levels, e.g. at a neutron dose of 10 mGy. RBE values derived with reference to gamma radiation will be appreciably higher than those assessed with regard to X rays, since the RBE of the first type of radiation is considerably smaller than unity. When the estimates are based on different assumptions, the same set of experimental data can result in widely differing RBE values. In reporting his own data on lung adenocarcinoma, Ullrich[28] derived an RBE of 18.5 on the basis of the linear slope coefficients for the neutron and gamma ray dose-response curves, whereas Fry[12] quoted an RBE of 60 on the basis of the same criteria. Assuming the inverse-square root dose-dependence, Ullrich inferred an RBE of 71 for a neutron dose of 10 mGy. The calculation of different RBE values from the same experimental studies implies that caution should be exercised in the interpretation of such data.

The studies performed by Shellabarger et al.[24] on mammary neoplasms in Sprague Dawley rats have indicated proportionality of RBE to the inverse square root of the neutron dose. Only a minor fraction of all mammary tumours observed in this study consisted of adenocarcinomas. However, the authors claim that the RBE-dose dependence for fibroadenoma alone and the combined group of fibroadenoma and carcinoma is also consistent for the more limited data pertaining to adenocarcinoma. In a subsequent experiment, Shellabarger et al.[25] investigated the effect of radiation and the administration of diethylstilbestrol (DES) in another rat strain. For ACI rats treated with DES, the RBE values are larger than those for Sprague Dawley rats at comparable neutron doses.

A listing of RBE values for neutrons with energies between 0.43 and 1 MeV is given in table 6. For the induction of myeloid leukaemia in mice, Mole[21] reported a quadratic dose term without a statistically significant linear dose component after X-irradiation. For these

Table 6. Relative biological effectiveness at a dose of 10 mGy of neutrons with energies 0.43–1 MeV for different endpoints

	RBE at 10 mGy
Lung adenocarcinoma in mice, a_1 neutrons/a_1 photons	18.5
$D_N^{-1/2}$, Ullrich[28]	71
Malignant transformation in hamster embryo cells Borek and Hall[2]	10–25
Myeloid leukaemia in mice, daily chronic irradiation Upton et al.[32]	16
Myeloid leukaemia in mice, acute irradiation, square root of a_1 neutrons/a_2 photons, Mole[21]	13
Myeloid leukaemia in mice Ullrich and Preston[30]	3
Fibroadenoma in Sprague Dawley rats, $D_N^{-1/2}$, Shellabarger et al.[24]	50
Adenocarcinoma in ACI rats treated with DES, $D_N^{-1/2}$, Shellabarger et al.[25]	100
Adenocarcinoma in WAG/Rij rats, Broerse et al.[5]	15
Fibroadenoma in WAG/Rij rats, Broerse et al.[5]	13
Fibroadenoma in Sprague Dawley rats, Broerse et al.[5]	7
Life shortening in mice, Thomson et al.[27]	15
Life shortening in mice, Covelli et al.[10]	12

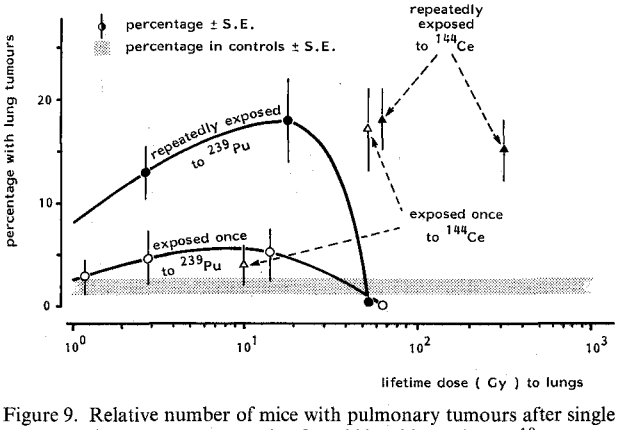

Figure 9. Relative number of mice with pulmonary tumours after single or repeated exposure to aerosols of Pu-239 oxide or Ce-144[19].

data, the RBE was calculated as the square root of the ratio a_1 for neutrons to a_2 for X rays. The RBE values for induction of mammary tumours [5] are based upon the linear dose-response curves for neutrons and X rays. Appreciably higher RBE values would be obtained, when the results of the gamma exposure shown in figure 8 would be used as a base line. It should be recognized, however, that such an increase in RBE will be caused by the lower efficiency of low-LET radiation rather than by an increase in efficiency of the neutron irradiation at low doses.

Effects of fractionation or protraction of the dose

When the radiation dose is administered in a number of fractions or at a reduced dose rate, the biological response is different from that obtained after single acute doses. For cell survival, the modifying processes include repair of sublethal damage, repopulation and re-distribution over the cell cycle. For tumour induction the nature of modifying mechanisms is not yet well understood, and it is rather difficult to interpret all experimental results obtained after fractionated or protracted exposures.

In general, for low-LET radiation the effectiveness is reduced with respect to the acute exposure when fractionation or protraction is applied. One of the few exceptions are the studies of Maisin et al.[20] which indicated that exposure to Cs-137 gamma radiation delivered in 8 fractions (interval 3 h) and 10 fractions (interval 24 h) would be more effective in causing leukaemias and all types of carcinomas and sarcomas in mice than a single session irradiation.

For high-LET radiation the effects of fractionation and protraction (see table 7) are different for the various tumour types, presumably because of the different mechanisms of tumour induction that are involved. For the induction of ovarian tumours in mice, Ullrich[29] observed that fission neutron irradiation was less effective when delivered at low dose rates in comparison with high dose rates. However, the mammary carcinogenic effect of neutrons was enhanced at low dose rates, a finding similar to that of Vogel and Dickson[34]. Studies on the induction of mammary carcinoma in WAG/Rij rats[5] after single and fractionated irradiations with X rays and 0.5 MeV neutrons indicate that for equal total absorbed dose the tumours appeared at approximately the same age. It should be noted that the experimental studies on mammary carcinogenesis are generally based on whole-body irradiations of the animals. The induction of mammary cancer can easily be modified by hormonal factors, and it might well be that specific endocrinological effects caused by the irradiation influence mammary tumour induction to a lesser extent when fractionated or protracted exposures are studied.

Table 7. The effect of fractionation or reduction in dose rate on tumour induction or longevity in experimental animals after high-LET irradiation

	Change in effectiveness*
Mammary tumours	
Ullrich[29]	+
Vogel und Dickson[34]	+
Broerse et al.[5]	=
Ovarian tumours	
Ullrich[29]	−
Pulmonary tumours	
Ullrich[29]	=
Little et al.[18]	=
Lundgren et al.[19]	+
Life shortening in mice	
Thomson et al.[27]	+
Maisin et al.[20]	=

* Enhanced (+), reduced (−) er equal (=) with respect to single- or high-dose rate irradiation.

The induction of lung cancer after single or protracted irradiation with α-particles was investigated independently in two species. Lundgren et al.[19] studied the effect of inhalation of Pu-239 oxide in mice after single or repeated exposure. Their results are presented in figure 5, together with incidence data of pulmonary tumours induced by exposure to aerosols of the β-emitting radionuclide Ce-144[13]. For similar cumulative doses from α-particles, an approximately four times greater incidence of pulmonary tumours was observed than for the single-inhalation exposure. The effect of dose rate on the induction of lung cancer in Syrian hamsters was examined by intratracheal instillation of Po-210[18]. Protraction of the α-irradiation over 120 days was slightly more carcinogenic at lower total lung doses but slightly less carcinogenic at higher doses, in comparison to an exposure limited to a 10-day period. The carcinogenic effect of a single-intratracheal instillation of Po-210 was markedly enhanced by subsequent weekly instillation of saline alone, emphasizing the importance of non-carcinogenic secondary factors in the expression of radiation-induced lung cancer.

Conclusions

The carcinogenic action of ionizing radiation has been demonstrated for a spectrum of endpoints in experimental animals. It has to be concluded, however, that the peculiarities of each tumour model are such as to preclude generalizations[31]. Furthermore, it is difficult to interpret tumour induction curves on the basis of simple mechanisms of action, in view of the complex interplay of primary and secondary factors. Physical factors, such as absorbed dose, and its temporal distribution and the linear energy transfer have an appreciable influence on the carcinogenic action.

For an appropriate evaluation of in vivo carcinogenesis studies, actuarial methods should be applied. It will be of scientific and practical interest to analyse the same sets of tumour induction data by different mathematical models.

Concerning the nature of dose-response relationships a general consensus can be reached about linear depen-

dence on the dose for high-LET radiation. For low-LET radiation, however, approximately pure quadratic dose-response curves have been reported for a number of end-points. Apparently, repair processes at the (sub)cellular level and systemic factors can modify the primary process of cancer initiation.

As a consequence of the results reported in subhuman primates, it can be recognized that there is a strong need to screen regularly for secondary tumours which may arise in patients who previously received high-dose total-body irradiation.

Within the range of neutron energies investigated, the highest RBE values are observed for neutron energies around 1 MeV. For the different types of radiation-induced malignancies observed in various species and strains, varying RBE values are derived. Based on the current evidence, it seems appropriate to adopt a maximum quality factor of 20 for neutrons with reference to X-rays.

In general for low-LET radiation, the sparing effect of fractionated or protracted irradiation is demonstrated. However, for high-LET radiations the animal studies do not provide an unambiguous answer. For the induction of pulmonary tumours after inhalation of α-emitting nuclides enhanced effects are observed at low-dose rates in comparison to single high-dose rate exposures. This finding which could have implications for the implementation of exposure limits for occupational and domestic situations, deserves further investigation.

Acknowledgment. The experimental studies have been supported by the Koningin Wilhelmina Cancer Foundation, the Dutch Preventiefonds and the Commission of the European Communities.

1 Bekkum, D. W. van, Broerse, J. J., Hennen, L. A., and Solleveld, H. A., The gene transfer-misrepair hypothesis of radiation carcinogenesis tested for induction of mammary tumours in rats. Leuk. Res. *10* (1986) 761–766.

2 Borek, C., and Hall, E. J., Induction and modulation of radiogenic transformation in mammalian cells, in: Radiation Carcinogenesis: Epidemiology and Biological Significance, pp. 291–302. Eds J. D. Boice Jr and J. F. Fraumeni Jr. Raven Press, New York 1984.

3 Bradle, E. W., Zook, B. C., Casarett, G. W., Deye, J. A., Adoff, L. M., and Rogers, C. C., Neoplasia in fast neutron-irradiated beagles. J. natl Cancer Inst. *67* (1981) 729–738.

4 Broerse, J. J., Bekkum, D. W. van, Hollander, C. F., and Davids, J. A. G., Mortality of monkeys after exposure to fission neutrons and the effect of autologous bone marrow transplantation. Int. J. Radiat. Biol. *34* (1978) 253–264.

5 Broerse, J. J., Hennen, L. A., and Zwieten, M. J. van, Radiation carcinogenesis in experimental animals and its implications for radiation protection. Int. J. Radiat. Biol. *48* (1985) 167–187.

6 Broerse, J. J., Hennen, L. A., and Solleveld, H. A., Actuarial analysis of the hazard for mammary carcinogenesis in different rat strains after X- and neutron-irradiation. Leuk. Res. *10* (1986) 749–754.

7 Broerse, J. J., Hennen, L. A., Klapwijk, W. M., and Solleveld, H. A., Mammary carcinogenesis in different rat strains after irradiation and hormone administration. Int. J. Radiat. Biol. *51* (1987) 1091–1100.

8 Chmelevsky, D., Kellerer, A. M., Lafuma, J., Morin, M., and Masse, R., Comparison of the induction of pulmonary neoplasms in Sprague-Dawley rats by fission neutrons and radon daughters. Radiat. Res. *98* (1984) 519–535.

9 Coggle, J. E., Lung tumour induction in mice after X-rays and neutrons. Int. J. Radiat. Biol. *53* (1988) 585–598.

10 Covelli, V., Coppola, M., Di Majo, V., Rebessi, S., and Bassani, B., Tumor induction and life shortening in $BC3F_1$ female mice at low doses of fast neutrons and X-rays. Radiat. Res. *113* (1988) 362–374.

11 Deeg, H. J., Prentice, R., Fritz, T. E., Sale, G. E., Lombard, L. S., Thomas, E. D., and Storb, R., Increased incidence of malignant tumors in dogs after total body irradiation and marrow transplantation. Int. J. Radiat. Onc. Biol. Phys. *9* (1983) 1505–1511.

12 Fry, R. J. M., Experimental radiation carcinogenesis: what have we learned? Radiat. Res. *87* (1981) 224–239.

13 Hahn, F. F., Lundgren, D. L., and McClellan, R. O., Repeated inhalation exposure of mice to $^{144}CeO_2$. II. Biological effects. Radiat. Res. *82* (1980) 123–137.

14 ICRP. Recommendations of the International Commission on Radiological Protection. ICRP Publication 26, Annals of the ICRP 1977 (3).

15 Kalbfleisch, J. D., and Prentice, R. L., The Statistical Analysis of Failure Time Data. John Wiley and Sons, New York 1980.

16 Kaplan, E. L., and Meier, P., Non-parametric estimation from incomplete observations. J. Am. Statist. Ass. *53* (1958) 457–481.

17 Kellerer, A. M., and Chmelevsky, D., Analysis of tumor rates and incidences – a survey of concepts and methods, in: Neutron Carcinogenesis, EUR 8084, pp. 209–231. Eds J. J. Broerse and G. B. Gerber. Commission of the European Communities 1982.

18 Little, J. B., Kennedy, A. R., and McGandy, R. B., Effect of dose rate on the induction of experimental lung cancer in hamsters by α radiation. Radiat. Res. *103* (1985) 293–299.

19 Lundgren, D. L., Gillett, N. A., Hahn, F. F., Griffith, W. C., and McClellan, R. O., Effects of protraction of the α dose to the lungs of mice by repeated inhalation exposure to aerosols of $^{239}PuO_2$. Radiat. Res. *111* (1987) 201–224.

20 Maisin, J. R., Wambersie, A., Gerber, G. B., Mattelin, G., Lambiet-Collier, M., Coster, B. de, and Gueulette, J., Life-shortening and disease incidence in C57Bl mice after single and fractionated gamma and high-energy neutron exposure. Radiat. Res. *113* (1988) 300–317.

21 Mole, R. H., Dose-response relationships, in: Radiation Carcinogenesis: Epidemiology and Biological Significance, pp. 403–420. Eds J. D. Boice Jr and J. F. Fraumeni Jr. Raven Press, New York 1984.

22 Preston, D. L., and Pierce, D. A., The effect of changes in dosimetry on cancer mortality risk estimates in the atomic bomb survivors. RERF TR 9–87. Radiation Effects Research Foundation, Hiroshima 1987.

23 Roesch, W. C. (Ed.), US-Japan Joint Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki. The Radiation Effects Research Foundation, Hiroshima, Japan 1987.

24 Shellabarger, C. J., Chmelevsky, D., and Kellerer, A. M., Induction of mammary neoplasms in the Sprague-Dawley rat by 430-keV neutrons and X-rays. J. natl Cancer Inst. *64* (1980) 821–833.

25 Shellabarger, C. J., Chmelevsky, D., Kellerer, A. M., and Stone, J. P., Induction of mammary neoplasms in the ACI rat by 430 keV neutrons, X-rays, and diethylstilbestrol. J. natl Cancer Inst. *69* (1982) 1135–1146.

26 Shimizu, Y., Kato, H., Schull, W. J., Preston, D. L., Fujita, S., and Pierce, D. A., Life span study report 11, part. 1. Comparison of risk coefficients for site-specific cancer mortality based on the DS86 and T65DR shielded kerma and organ doses. Technical Report, RERF TR 12–87. Radiation Effects Research Foundation, Hiroshima, Japan 1987.

27 Thomson, J. F., Williamson, F. S., and Grahn, D., Life shortening in mice exposed to fission neutrons and gamma-rays. Radiat. Res. *104* (1985) 420–428.

28 Ullrich, R. L., Tumor induction in BALB/c female mice after fission neutron or gamma irradiation. Radiat. Res. *93* (1983) 506–515.

29 Ullrich, R. L., Tumor induction in Balb/c mice after fractionated or protracted exposures to fission-spectrum neutrons. Radiat. Res. *97* (1984) 587–597.

30 Ullrich, R. L., and Preston, R. J., Myeloid leukemia in male RFM mice following irradiation with fission spectrum neutrons or gamma rays. Radiat. Res. *109* (1987) 165–170.

31 UNSCEAR, 1986, Genetic and Somatic Effects of Ionizing Radiation, United Nations Scientific Committee on the Effects of Atomic Radiation. United Nations, New York 1986.

32 Upton, A. C. Randolph, M. L., and Conklin, J. W., Late effects of fast neutrons and gamma-rays in mice as influenced by the dose rate of irradiation: induction of neoplasia. Radiat. Res. *41* (1970) 467–491.

33 Upton, A. C., Radiobiological effects of low doses. Implications for radiological protection. Radiat. Res. *71* (1977) 51–74.

34 Vogel, H. H., and Dickson, H. W., Mammary neoplasia in Sprague-Dawley rats following acute and protracted irradiation, in: Neutron Carcinogenesis. EUR 8084, pp. 135–154. Eds J. J. Broerse and G. B. Gerber. Commission of the European Communities 1982.
35 Yochmowitz, M. G., Wood, D. H., and Salmon, Y. L., Seventeen-year mortality experience of proton radiation in Macaca mulatta. Radiat. Res. *102* (1985) 14–34.
36 Zwieten, M. J. van, The rat as animal model in breast cancer research. Martinus Nijhoff Publishers, Dordrecht/Boston 1984.

0014-4754/89/010060-10$1.50 + 0.20/0

Radiation embryology

C. Michel

Strahlenbiologisches Institut der Universität Zürich, August Forel-Strasse 7, CH-8008 Zürich (Switzerland)

Summary. Prenatal development, characterized by intensive cell proliferation, cell differentiation and cell migration, shows a high radiosensitivity. Therefore, radiation exposure of embryos and fetuses is of great concern for radiological protection and human health. Irradiation during gestation can cause death, growth disorders, malformations, functional impairment and malignant diseases in childhood. These effects are strongly dependent on the developmental stage at exposure and on the radiation dose. The first trimester of pregnancy is regarded as the period with the highest risk for malformation and cancer induction. The developing nervous system shows a special susceptibility to ionizing radiation over a long period and is therefore of great significance for risk estimation. Knowledge about radiation effects on prenatal development has been derived from animal experimentation and from the exposure of human embryos. There is evidence that doses between 1 and 10 cGy may lead to developmental anomalies and that the radiation response can be modified by additional factors.
Key words. Prenatal development; ionizing radiation; in vitro tests; animal experiments; human studies; low dose effects; risk estimation.

Introduction

In considering the potential health risks of ionizing radiation, developmental effects of exposure in utero deserve the same attention as the postnatal induction of cancer or genetic damage. This statement is based on the particular sensitivity of embryonic and fetal cells or tissues to radiation. In addition, there are two recent findings of possible concern regarding the biological consequences of low-level exposure. One is the radiation-related mental deficit among the in utero exposed survivors of the atomic bombing of Hiroshima and Nagasaki[63]. The other is the excess of lymphoid leukemia in young children in the vicinity of nuclear installations in England[14].

Although the deleterious effects of ionizing radiation on the developing embryo or fetus have been recognized since the beginning of this century, it was only in 1929 that Goldstein and Murphy[18] first comprehensively reviewed serious disturbances of the central nervous system in children of mothers receiving therapeutic pelvic irradiation during pregnancy.

While in the earlier experimental work relevant physical and biological parameters were missing, Job et al.[27] specified for the first time in 1935 both the dose of X-rays used and the developmental stage of the rat embryos during exposure.

Meanwhile, extensive investigations have been conducted on the biological effects of prenatal irradiation. Especially over the last 10 years this subject has attracted considerable interest as indicated by several reports and monographs[3, 13, 19, 20, 24, 67, 75, 77] from where most of the present information is derived. During this time numerous studies also contributed to a better understanding of the mechanisms of radiation-induced embryotoxicity. The purpose of this article is to summarize the current knowledge in radiation embryology and to present data which could be useful in evaluating possible risks from low level exposure.

1. Basic considerations on prenatal radiation effects

The principal effects of radiation on the mammalian conceptus are: embryonic, fetal or neonatal death, malformations, growth retardation, postnatal functional impairment and cancer induction. It is striking that besides these damages a normal development of irradiated embryos or fetuses has also been observed, indicating an effective recovery and compensation capacity of embryonic tissues. In considering the consequences of prenatal exposure to various forms of ionizing radiation, the following variables are of paramount importance: (a) the stage of development at the time of exposure; (b) the dose and dose distribution; (c) modifying factors.

The division of the intrauterine development into the preimplantation phase, the period of major organogene-