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Radiation embryology

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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.

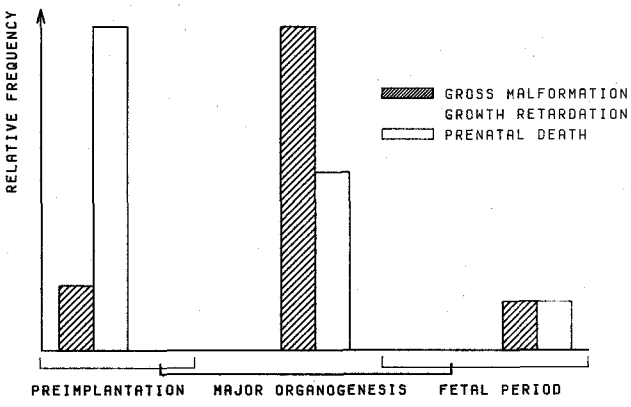
Meanwhile, extensive investigations have been conducted on the biological effects of prenatal irradiation. Especially over the last 10 years this subject has attracted considerable interest as indicated by several reports and monographs[3, 13, 19, 20, 24, 67, 75, 77] from where most of the present information 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-

Table 1. Relative incidences of developmental anomalies following intrauterine irradiation at various stages of development (from Fritz-Niggli[16], modified).



Mouse:	0–5	6–13	14–19.5
Rat:	0–7	8–15	16–21.5
Rabbit:	0–5	6–15	16–31.5
Man:	0–8	9–60	61–270

sis and the fetal phase is very suitable for the analysis of radiation effects, since each phase shows a characteristic pattern of damages. Table 1 schematically summarizes the so-called classic actions of radiation on developing mammals together with the approximate duration of phases for some species. Taking into account that the developmental processes occurring during these periods are basically alike, a cautious extrapolation of animal data to man seems justified at least in qualitative respects[59]. Since most of the data come from experiments with rodents, such an extrapolation has a significant bearing for the assessment of health risks following prenatal irradiation.

Dose and dose distribution of external and internal exposure strongly influence the incidence and severity of prenatal effects. In most studies, fractionated or protracted irradiation were less effective than acute exposure. Whereas the absorbed energy from external exposure to X-rays or γ-rays is uniformly distributed, an unequal dose delivery may result in the embryo or fetus from the maternal uptake of radioactive isotopes. In the evaluation of developmental effects by radionuclides, various physical and chemical characteristics as well as their metabolic behavior must be considered. Due to the complex processes, such as placental transfer and biokinetics (effective half-life) of radionuclides in developing tissues, the information about dose-effect relationships is still scarce[77]. However, the effects observed so far are not specific to internal irradiation but comparable to those related to external exposure.

Among other factors known to modify the embryotoxic action of radiation, sensitizing and protecting drugs are of basic interest to radioembryological research and risk estimations in man. Data from co-insult studies are needed to establish whether the current dose limits and recommendations are valid also for combined treatments. Experimental studies of combination effects involve various parameters (doses, route and sequence of application) and are therefore difficult to perform[71]. To our knowledge, there is only one report on the possible interaction between drugs and X-rays in human development. However, the observed increases in the relative risk of childhood cancer after joint treatments could also be associated with other factors[28].

2. *Radiation effects in the preimplantation period*

The preimplantation phase begins with the fertilization and ends with the implantation of the embryo into the uterine mucosa. During these few days of initial development (table 1) cell proliferation is the dominant event and therefore the main target for radiation injury.

2.1 In vitro studies

Techniques of embryo culturing in vitro represent a sensitive model system for radiobiological studies. They are also a very useful support for the data obtained in vivo, especially with regard to the mechanisms of radiation damage. Numerous biological end-points have been used in in vitro systems, e.g. DNA amount; chromosomal aberrations; micronuclei formation; G_2-block; cell cycle progression; proliferation rate; cell death and embryonic death; blastocyst formation and recovery processes.

The following principal results were obtained (Unscear-Report[77] unless otherwise indicated):

The pronucleus stage was found to be most radiation-sensitive, revealing a statistically significant enhancement of the preimplantation loss at a dose of 25 cGy X-rays. This particular sensitivity could be due to the dynamic events occurring following sperm penetration such as DNA synthesis, cell volume increase and fusion of the two pronuclei[78].

In the incidence of micronuclei, sparsely ionizing X-rays led to a linear relationship, whereas densely ionizing neutrons induced a steeper slope of the dose-effect curve. The enhanced frequency of micronuclei after neutron exposure can be explained with the higher number of double-strand breaks of DNA compared to X-irradiation. The formation of micronuclei leads to cells containing a hypoploid DNA content and may cause disturbances of cell proliferation, cell and embryonic death[47].

Exposures of the pronucleus stage of mouse eggs to tritium β-particles, ^{60}Co γ- or X-rays (10–40 cGy) resulted mainly in chromosomal fragments, indicating that internal and external radiations elicit similar DNA damage[32]. The dose-response relationship for the three types of radiation was linear. β-particles were more effective than γ- or X-rays as also shown with early mouse embryos, choosing blastocyst formation as the end-point.

When the effects of tritiated water (HTO) and tritiated thymidine on the development of 2-cell mouse embryos were compared the tritiated amino-acid was found to be extensively more effective (by a factor of about 1000) than HTO. This difference in effectiveness was explained

by β-ray-induced damage in the DNA due to the uptake of [^{3}H]thymidine [72].

Müller et al.[50] incubated two-cell stages of the mouse (in late G_2) with [^{3}H]thymidine and [^{3}H]arginine and scored for morphological development and formation of micronuclei. At the two levels of radioactivity used (370 Bq/ml and 925 Bq/ml medium) arginine generally was more efficacious than thymidine. This observation was attributed to the faster uptake of arginine and its immediate incorporation into proteins.

From the few transplantation experiments performed so far, it can be concluded that radiation-induced cell death in early embryos was compensated by the enhanced proliferation of surviving cells. The failure of gross malformations after transplantation of exposed embryos into foster mothers may signalize a complete recovery from radiation damage or resistance of the early phases of development to the teratogenic action of radiation.

The combined action of various environmental chemicals (including heavy metals) with ionizing radiation was reviewed by Streffer[70] and Müller and Streffer[48, 51]. Attention should be paid to the findings that relatively low concentrations of lead chloride or actinomycin potentiated the radiation effects and that caffeine (at high concentrations) considerably enhanced the radiation risk[49]. However, most of the substances tested (e.g. phenols, sodium nitrite, sodium sulfite, and ethidium bromide) showed an additivity of the single effects.

In general, more information is needed to judge the predictive value of in vitro testing with regard to radiation-induced prenatal toxicity in man.

2.2 In vivo experiments

Early radioembryological studies with mice revealed that the effects after exposure in the preimplantation period were basically different from those following treatment during organogenesis or fetogenesis. Embryonic death before or shortly after implantation is the most prominent response to irradiation during the early stages, whereas the proportion of malformation and growth retardation among survivors is smaller (table 1).

The highest sensitivity in relation to the lethal action of X-rays was found at 0, 2 and 3 days post conception (p.c.) in mice[75], i.e. during the early pronuclear stage, at the beginning of the second cleavage and at the blastocyst stage, respectively. The estimated values of LD 50 (lethal dose for 50% of the embryos) during these stages are in the order of 1 Gy.

A significant increase in embryonic mortality can be observed at a dose of 5 cGy given on day 0.5 of mouse gestation. From dose-effect studies on day 0.5 a linear progression analysis was performed leading to an increment of embryonic loss of about 1% per cGy. Regarding the mechanisms causing preimplantation death, chromosomal damage, formation of micronuclei ('subnuclei') and aneuploidy are regarded as the main factors. It appears that embryonic killing results as a direct action on the conceptus and is not induced through placental or maternal damages[75].

A series of papers deal with the question whether irradiation during preimplantation represents an 'all or nothing' effect, e.g. whether the early embryo either dies or develops normally. Among the teratogenic effects induced during the first day of development in mice the following were described: exencephaly, polydactyly, cataract, and gastroschisis[56, 75]. Although the frequencies of these malformations were much lower than those for preimplantation death, the findings are of exceptional interest. Not only the very early time of induction, but also the remarkably low dose levels in case of polydactyly and exencephaly (5 cGy) must be considered when asking for the lowest teratogenic dose in animal experimentation (table 2).

Significantly enhanced frequencies of growth-retarded mouse fetuses were found after exposure of pronuclear stages to negative pions with 13.5 cGy[37] and after X-irradiation of two-cell stages with 0.5 Gy[34].

In other experiments, no teratogenic effects and no increased rates of growth disturbances or reduced postnatal survival were observed[57, 61, 75].

In essence, the results of in vitro and in vivo studies are comparable in qualitative respects, demonstrating preimplantation death as the main response to radiation. Secondly, there are some indications for a teratogenic action also during the earliest phases of mammalian development; and thirdly, no estimates concerning the radiation risk during the first few days of human development exist as yet.

3. Radiation effects in the organogenesis

The organogenetic period in mice lasts from day 6 to day 13 p.c. and in the rat from day 8 to day 15, whereas in the human pregnancy the duration of this stage is much

Table 2. Lowest dose levels for some teratogenic effects in mice and rats.

	Type of damage	Day p.c. with maximal sensitivity	Lowest dose[a] (cGy)	Reference
Mouse	Skeletal anomalies	7 7.5	5 5	30 25
	Exencephaly	8	5	59
	Hydrocephaly, spinal cord flexion	8	25	77
	Microphthalmia	8	13.5 1 (peak pions)	35 36
	Anophthalmia	7.5	100 40 (1.2 MeV neutrons)	15
Rat	Microphthalmia, anophthalmia	8;9	25	33, 75
	Brain and spinal cord malformation	9	50	75

[a] X-rays unless otherwise indicated.

longer (days 9–60). Generally, the organogenesis represents the phase of mammalian development which is most intensively studied in radioembryological research. The literature is so vast that it is impossible to provide a comprehensive account on all the published data. For more complete information, the reader is referred to various monographs and reviews [3, 6, 21, 24, 31, 67, 75, 77].

Soon after implantation in the uterus, the embryo becomes markedly susceptible to radiation-induced teratogenic effects. Gross malformations in the central nervous system, skeleton, extremities, viscera, eyes and other organs are the most characteristic responses in experimental animals irradiated during the major period of organ formation. In man, microcephaly, mental retardation and microphthalmia were the most common damages in children after exposure in utero due to radiotherapy of the mother [3] or atomic bomb explosions [4, 63]. Besides the teratological changes, pre- and postnatal death and overall growth defects are also associated with irradiation during organogenesis.

3.1 Lethality

The maximum susceptibility for prenatal death is found on day 7.5 in mice and hamsters and on day 8.5 in rats [75]. Extensive studies by Jacobsen [25] resulted in a linear dose-effect curve for resorption incidences after exposure to 5, 50 and 100 cGy X-rays on day 7.5 of mouse gestation. Five cGy was the lowest effective dose in the animal strain used leading to a statistically significant enhancement of the resorption rate. However, other mouse data [15, 41] showed no marked increases in the resorption frequency with doses up to 1 Gy given on day 8 p.c.

It can be concluded that there are large differences in the susceptibility among animal strains and great variations in the response depending on the developmental stage and irradiation conditions. Secondly, there are no reliable data in man referring to the lethal effects of radiation during organogenesis [75].

3.2 Malformations

In radiation teratogenesis much attention is spent on the question concerning the lowest teratogenic dose. The compilations published so far on this topic comprise dose-levels from 1 to 50 cGy.

Table 2 selectively reviews the lowest teratogenic doses with respect to some common effects observed in mice and rats after external irradiation. It is emphasized that this presentation does not include negative findings which should receive the same importance as positive ones [44]. Radiation-induced skeletal anomalies show an interesting time specificity, since the early stage (day 7) is more sensitive than the main period of skeleton formation (days 10–13). The lowest dose tested (5 cGy) induced defects mainly in the vertebral column, sternum, ribs, skull and paws [25, 30]. Exencephaly results from a non-closure of the neural folds at the mid-brain region and was described as an anomaly which may be induced by X-rays without a threshold [60]. This statement is refuted by recent findings (15, 40), showing heavy brain damage at 40 and more cGy X-rays or 20 cGy of 1.2 MeV neutrons only. Hydrocephaly and spinal cord abnormalities, such as flexion and hydromyelia were found after exposure to 25 cGy on day 8 [75].

Concerning ocular malformations, the frequency of microphthalmia was 10% after exposure of mouse embryos on day 8 to 13.5 cGy X-rays, or 21% after the same dose of negative pions [35]. Irradiation with peak pions was effective even at a dose of 1 cGy, revealing a 3-fold increase in the spontaneous rate of microphthalmia. Microscopic analyses of optic primordia following exposure of mouse embryos during early organogenesis showed that recovery processes and changes in the necrosis pattern and proliferation activity are decisive events in the formation of eye defects [1]. Anophthalmia has been produced at low frequencies with doses of 1 Gy X-rays or 0.4 Gy neutrons [15]. In rats, the few data indicate that doses between 25 and 50 cGy are needed to produce eye, brain or spinal cord defects [33, 75].

From the data of Okamoto et al. [53] it can be assumed that even lower doses of neutrons than 80 cGy may produce cardiovascular malformations. Other studies with neutrons [8, 12] have shown that qualitatively the same types of malformations were induced as with γ- or X-rays. Quantitatively, neutrons were 2–4 times more effective than sparsely ionizing radiations.

In spite of the death of complete dose-effect series in animals, it seems that the most teratological effects have a curvilinear relationship with the dose. Linear dose-effect curves without distinct thresholds were claimed for some skeletal anomalies [25], whereas a complex non-linear slope, containing a plateau region between 25 and 50 cGy, was observed in other studies [30, 39, 40].

In man, several types of radiation-induced abnormalities such as small head size, mental deficiencies, eye anomalies, hydrocephaly, spina bifida and skeletal defects have been enumerated [3]. Small head size (often denoted as microcephaly) is particularly associated with exposure during the 6–11th gestational week and in about 10% of the cases combined with mental retardation. Both effects are thoroughly analyzed in the light of developmental processes by a task group of the International Commission on Radiological Protection [24]. The main conclusions concerning severe mental retardation and small head size will be presented in section 4.

The great number of papers reporting on cellular changes which may be involved in the genesis of central nervous system malformations or other organ defects following external or internal irradiation are discussed in the monographs mentioned in the introduction and by others [22, 31]. Among the most important mechanistic factors are: disturbances on the macromolecular level (e.g. DNA, RNA, proteins and membranes); impairment of the energy metabolism; chromosomal aberrations; prolongation of the cell cycle time; cell inactivation; cell

death; as well as influence on induction, coordination and organization processes, cell differentiation, migration and cell positioning. According to Brent [6] it is difficult to determine which mechanisms are primarily operative at the specific stages of gestation where corresponding embryonic disorders are induced.

3.3 Growth disorders

The stages of major organogenesis are probably more sensitive to growth disorders than to the teratogenic action of radiation. However, due to effective regulatory powers of embryonic tissues [29] some of the growth deficits may be repaired during the intrauterine development. Threshold doses for radiation-induced growth retardation are strongly species- and age-dependent: in 8-day-old rat embryos exposure to 20–40 cGy had no significant effect on postnatal growth [26], whereas in mice a transient slight increase in the rate of retarded fetuses was present after irradiation with 1 or 13.5 cGy on day 8 [35, 36].

Human data for postnatal growth after in utero exposure to atomic bombs or diagnostic procedures showed that the growth-retarding effect of radiation is most pronounced during the second month of pregnancy [75]. As yet, no proper dose-effect relations are available and the influence of other factors (nutrition, infections) is not clarified. As a minimum dose for non-recuperable growth retardation a range between 25 and 50 cGy is estimated for the human embryo.

3.4 Functional changes

Animal offsprings have been tested for locomotor coordination, activity, maze learning, avoidance response, audiogenic seizure and other neurological functions in order to evaluate behavioral abnormalities. Data on significantly decreased performances after in utero exposures of rats in the dose range of 50–100 cGy are reviewed by Hicks and D'Amato [22]. However, significant changes in the learning ability are reported after applying four daily doses of 1 cGy on days 6–9 of rat gestation [5]. More research efforts are needed to overcome uncertainties in this field and to determine radiation risks of low doses with respect to behavioral changes.

Concerning the responses of primordial germ cells, there is no evidence that embryonic gonads are more sensitive to the mutagenic action of radiation than adult testes or ovaries [6].

3.5 Tumor induction

The so-called Oxford survey, published by Stewart et al. [69], was the origin for extensive research programs and stimulating scientific discussion. The remarkable aspect of this large retrospective study is the excess of leukemia and other cancers in children prenatally exposed in the course of diagnostic X-ray examination of their mothers. There is a suggestion that the relative risk of cancer is higher for children irradiated in the first trimester of pregnancy [3]. Brent [6] estimated that doses between 1 and 2 cGy may increase the risk for developing leukemia in offsprings by a factor of 1.5 to 2 compared to the spontaneous incidence. Risk factors for tumor induction and other developmental anomalies are listed in table 3.

Table 3. Risk estimates in humans for radiation damage after exposure in utero.

Exposure time (Pregnancy)	Criteria	Risk of induction	Reference
8–15 weeks	Severe mental	$4 \cdot 10^{-3}$ (0.4%)/cGy	55
16–25 weeks	retardation	$1 \cdot 10^{-3}$ (0.1%)/cGy	24, 77
0–8 days	Preimplant. loss	10 /Mio/cSv/year*	23
0–17 weeks	Malformation	10 /Mio/cSv/year*	
Whole pregnancy (1st trimester with higher risk)	Malignant, fatal disease	dose range: 0.2–20 cGy	
		20 /Mio/cGy/10 years	75
		23 /Mio/cGy/year	68
		53 /Mio/cGy/year	3
		200–250 /Mio/cGy	75
		230 /Mio/cGy	77

* Risk estimates for a female-employed population exposed to 1 cGy per year.

The interesting finding of Stewart and her coworkers was confirmed by other studies as summarized by Fritz-Niggli [16]. The latest report of the United Nations Scientific Committee on the Effects of Atomic Radiation [77] covers in depth the incidence of malignancies following external and internal exposure in utero. According to the Committee's judgement there is a causal association between exposure in utero to diagnostic X-rays and an increased risk of cancer during childhood. This consideration is aligned with practical safety aspects and thought as a measure of prudence. However, it is recognized that the nature of this association remains unsolved, relying mainly on the following facts: 1) no relationship could be observed so far between prenatal irradiation and enhanced cancer risks in the survivors of Hiroshima and Nagasaki; 2) there exist two great prospective studies showing no increased tumor incidence following diagnostic exposures in utero [10, 65]; 3) the medical conditions which demanded the use of X-rays might themselves be associated with the subsequent occurrence of cancer [54, 74].

Independent reports provided data on cancer mortality in England and Wales and discussed its possible association with proximity to nuclear installations and other factors. The most recent paper [14] did not show a generalized increase in cancer mortality in zones near the installations. On the contrary, the death risks of persons aged 0–24 years from some tumor types were even lower than those in comparable control areas. However, the relative risk for lymphoid leukemia in children aged 0–9 years was significantly increased in the nearest zones of the nuclear plants. Prenatal induction of this disease must be taken into account. The present knowledge is insufficient to convict radiation from radioactive discharges as the causing agent or to unveil other reasons for this disquieting finding. Thus, a comprehensive study of children

exposed near Chernobyl could provide more insight into the assessment of cancer risks.

Regarding the problem of cancer induction in man, animal data failed to confirm a higher sensitivity of intrauterine stages to radiation cancerogenesis. The results from numerous experiments with mice, rats and dogs are not uniform and show considerable differences in the tumor incidence of various organs [77]. Referred to the dose, a cancerogenic effect may be limited to a narrow range because of cell inactivation and cell killing as competitive reactions. Schmahl [62] reported that 1 Gy is the most effective dose with respect to cancer induction and the liver the most sensitive target in the mouse fetus on day 12. Generally, rats tend to be more frequently affected than mice, and dogs may be even more susceptible to the cancerogenic action of radiation than rodents [66]. Radionuclides such as ^{3}H, ^{14}C, ^{32}P, ^{90}Sr, ^{131}J, ^{137}Cs, ^{239}Pu and others also have the potential for carcinogenic efficacy [77]. Their metabolic behavior and dose delivery to embryonic or fetal tissues differs from the situation in adult organisms, impeding therefore comparative risk estimates between pre- and postnatal stages.

4. *Radiation effects in the fetal period*

Fetogenesis adjoins the organogenetic period without distinct demarcations and therefore the sensitivity for some radiation damage spreads on both phases. This is true especially for the central nervous system (CNS) which displays a long time of susceptibility to radiation. For small head size and severe mental retardation in children, the weeks 6–11 p.c. and weeks 8–15 p.c. respectively represent the most critical phases. These developmental stages are characterized by the formation and maturation of the cortical plate, exhibiting two waves of proliferation and migration of neuronal cells. It is assumed that interferences with cellular phenomena such as proliferation, necrosis, migration, differentiation and positioning are responsible for the two typical defects in the CNS mentioned before [24].

Small head size and severe mental retardation occurred at the very low dose range of 1–9 cGy [55] and hence are of profound interest for radiological protection in man. Whereas the frequencies of both brain damages in the lowest dose category were insignificant, the incidences at higher doses (>10 cGy) showed significant differences to the control. Severe mental retardation following irradiation in the 8–15th week after fertilization appears to be linearly related to the dose without a threshold [64]. Within this critical period, mental retardation could be denoted as a stochastic phenomenon though this effect may be based on a multicellular reaction mechanism, e.g. only the damage to a number of cells leads to the observed response [17]. This stochastic model was questioned recently by Mole [46], arguing that severity in mental impairment represents a nonstochastic form of radiation effect. In addition, it is emphasized that other factors (e.g. infections, nutritional deficiencies) should also be considered in the risk analysis. The same author asks whether severe mental retardation may be an inherited anomaly, i.e. related to X-chromosomal damages [45]. In this case at least some of the observed retardations would not have been caused by irradiation after conception.

In what concerns risk estimation, the corresponding risk for severe mental retardation after irradiation during the weeks 8–15 p.c. is about 0.4% per cGy, assuming a linear dose-response relationship (table 3). Exposure during the weeks 16–25 seems to result in a linear-quadratic dose-effect curve with a four times less risk per unit dose. There was no increased risk at 0–7 weeks or after the 25th week [24]. In the control group, the probability for the occurrence of this serious cerebral defect at Hiroshima and Nagasaki was 0.8%.

New information is available with regard to lesser degrees of neurological deficits comprising changes in scholastic performances [63]. The preliminary data confirm the period of 8–15 weeks as being maximal sensitive also for the diminuition in intelligence. In two other studies, no evidence for mental retardation was reported following in utero exposure on the occasion of diagnostic X-rays examination [43, 52].

Most studies with nonhuman primate fetuses were performed at late stages, i.e. not in the period of maximum radiosensitivity of the brain cortex [24]. Neurological effects such as impairment of visual orientation, nervous activity and learning ability represent the most prominent findings. Experiments with rodents dealt with sensory, learning and motor functions during the so-called brain growth spurt. Doses in the range of 25 to 200 cGy not only led to functional disorders but also to gross structural brain damages (reduced cortex thickness, formation of rosettes). Moreover, loss of neuronal cells, changes in the proliferation pattern and metabolic activities as well as alterations of the fine structure of neuronal cells were reported. The literature pertaining to these radiation responses is reviewed in the UNSCEAR reports [75, 77] and by Konermann [31].

5. *Modification of prenatal radiation effects*

Possible interactions between radiation and chemicals or other factors have received increasing interest over the past few years. From the standpoint of health risks, the aspect of combined effects in the sensitive mammalian conceptus is meaningful because many interactions might occur in our environment [76].

Various chemicals were tested in vivo for their ability to modify radiation effects, taking as end-points the embryonic or fetal lethality, body weight reduction, sex ratio, and the teratogenic or cancerogenic action [77]. Papers describing synergistic interaction between radiation and chemicals such as chloroquine, caffeine, cortisone,

iodoacetamide, lucanthone, tetracyclines, urethane, 5-azacytidine, and trypan blue have been reviewed [34, 75, 77]. Misonidazole, a hypoxic cell sensitizer, potentiated teratogenic and growth retarding effects of electrons, X-rays and negative pions [38, 39]. Recent data on the combined effects of radiation and vindesine, an oncolytic drug, showed dose- and time-dependent radiosensitization concerning teratogenic responses in mice [2].

Potentiation of the teratogenic effects of γ-radiation by microwave-induced hyperthermia was observed in rat fetuses following treatment on day 10 of gestation [58].

Radioprotective actions were reported with serotonin, mexamine, and a number of sulfhydryl compounds, e.g. cysteamine, cystamine, mercaptopropionylglycine [11, 75, 77]. Other studies provide evidence for radioprotection by selenium [9] and vitamin E [73]. Anesthesia and hypoxia also protected against radiation-induced developmental abnormalities in mice as reviewed in the UNSCEAR report 1977 [75]. Interesting data were obtained after combined exposure of mouse embryos to heavy metals [42] or urethane [7] and radiation, revealing antiteratogenic and anticarcinogenic effects of X-rays in chemically treated mice.

As far as the mechanisms are concerned reactions with free radicals and SH-groups and/or metabolic effects such as interference with cell proliferation and recovery may be the most important factors in radiomodification. However, current knowledge should be improved in order to gain further insight into situations where interactions are possible.

6. *Risk estimation and research needs*

In addition to the risk factors listed for individual prenatal radiation effects in table 3, the overall risk per cGy was estimated in the UNSCEAR-Report 1986 [77]. The detriment calculated includes lethality, malformations, severe mental retardation and tumors. If one cGy is delivered over the whole human pregnancy the committee derived a risk for developmental anomalies of less than 0.2%. By comparison, the natural prevalence of malformations at birth was 6%.

For the protection of the embryo or fetus from ionizing radiation, dose limits of 0.5 or 1 cGy were set by national and international committees. Whether these levels provide sufficient protection for any prenatal stage remains the subject for future discussions. In this respect, collecting epidemiological data and further experimental work at low doses (below 10 cGy) are of highest priority [77]. Especially the knowledge about effects after exposure to internal radiation sources must be improved by performing comparative studies on different animal species. Finally, methods and techniques should be applied, which allow to discover subtle morphological and functional changes at various time intervals after exposure to radiation or combined modalities of treatment.

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Full Papers

Lipid characterization and ^{14}C-acetate metabolism in catfish taste epithelium [1]

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Summary. The catfish, *Ictalurus punctatus* is an important model system for the study of the biochemical mechanisms of taste reception. A detailed lipid analysis of epithelial tissue from the taste organ (barbel) of the catfish has been performed. Polar lipids account for 62 ± 1% of the total, neutrals for 38 ± 1%. Phosphatidyl-cholines, serines and ethanolamines are the major constituents of the polar fraction. Plasmalogen concentration is high relative to that of non-neural tissues. [^{14}C]-Acetate is incorporated into cell lipid fractions after incubation of barbel tissue at 37 °C for 60 min. Percentage amounts of most lipids change with time during this in vitro incubation. The phospholipids are the most metabolically active fractions. This work yields information for continuing reconstitution experiments and indicates that the taste epithelium of this important model system is a metabolically active tissue capable of supporting lipid turnover/synthesis.
Key words. Taste; phospholipid; plasmalogen; ^{14}C-acetate; epithelium.

While the receptor events in taste are beginning to be understood [2, 3], the molecular details of reception and transduction for various qualities are still incompletely described. For example, responsitivity to salt is apparently largely due to the presence in the taste cell membrane of nonvoltage gated (and in most cases, amiloride-sensitive) epithelial channels [4–7]. These channels have yet to be isolated and studied in vitro, and their stability and conformational characteristics are not known. Response to sour (acidic) stimuli may be mediated by a proton block of outward potassium current [8]. There is suggestive evidence for receptor-mediated responses to sweet stimuli [9–11], but molecular details are not known. Receptor mechanisms for bitterness have not been explored in depth [3].

Much of the slow progress in the past in understanding the molecular mechanisms of taste stemmed from the lack of suitable animal models. Recently, however, the amino acid taste system of the catfish has been exploited as a model. The taste system of this animal shows a high degree of sensitivity and specificity for amino acids [13–16]. The barbels of the catfish are especially dense in taste buds particularly on the leading and trailing edge of the barbel (the barbel is elliptical in cross-section) where the sensory nerves traverse the length of the organ. Progress is being shown with this model [15, 16], and purification and reconstitution of some receptors is a definite possibility. In fact, reconstitution of isolated plasma membranes into phospholipids at tips of patch electrodes has been reported, and stimulus-activated cation channels were observed [17]. It is, therefore, imperative that the amount and reactivity of the membrane lipids be assessed in order to provide a basis for the experiments that will seek understanding of the molecular details of reception and transduction. Consequently, we report here the lipid composition of the taste epithelia of the main taste receptor organ of the catfish – the maxillary barbels – and the incorporation of the metabolic substrate, acetate, into the major lipid classes.

Materials and methods

Solutions. Krebs-Ringer bicarbonate solution was made to the following component concentrations: NaCl, 120 mM; KCl, 4.75 mM; $CaCl_2$, 1.2 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 0.12 mM; $NaHCO_3$, 25.9 mM; pH 7.4. Lipid standards were purchased from Applied Sciences, State College, PA and tested for purity by chro-