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LES EFFECTS D'UNE IRRADIATION PRÉNATALE

INVLOED VAN STRALING OP EEN ORGANISME IN ONTWIKKELING

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Les effets d'une irradiation prénatale

Invloed van straling op een organisme in ontwikkeling

SOMMAIRE   **INHOUD**

Effects of radiation on the developing organism

P. Jacquet
Laboratory of Radiobiology,
CEN/SCK Mol

Summary

The embryonic development can be subdivided into three periods: (1) the preimplantation period, which extends from fertilization to the time when the embryo attaches to the wall of the uterus; (2) the organogenesis period, during which the major organs are developed; and (3) the foetal period, during which growth of the newly formed organs takes place. Experiments performed in laboratory animals have shown that each of these periods is characterized by a particular sensitivity to ionizing radiation or other toxic agents. Thus, it is widely believed that an irradiation during the preimplantation period may essentially result in the death of the embryo. Irradiation during the phase of organogenesis may characteristically result in the production of a variety of congenital anomalies. The induction of anomalies depends on the number of damaged cells in the forming organ and will not occur below a certain threshold (100 mGy in the mouse). Irradiation during the foetal period can induce anomalies in the development of the tissues and general or localized growth retardation. The growth retardation induced during this period frequently persists during all the extra-uterine life.
In man, radiation-induced malformations of body structures other than the central nervous system are uncommon. However, exposure of the human conceptus during the last part of pregnancy, the foetal period, may lead to severe mental retardation associated or not with microcephaly (decrease in head diameter). Data obtained in children who had been exposed *in utero* to the A-bombs in Hiroshima and Nagasaki showed that the probability of SMR (severe mental retardation) is essentially zero for an exposure occurring at less than 8 weeks of gestational age, is maximal for an exposure during the period 8-15 weeks (95 % lower limit of the threshold : 0.06-0.31 Gy) and decreases during the period 16-25 weeks (95 % lower limit of the threshold: 0.28 Gy).
In addition to these effects, some studies have established a link between irradiation *in utero* and the subsequent development of leukaemia and other childhood malignancies. Although far from being proved, this risk is now accepted by the most important scientific committees on account of prudence (2.78 %/Gy).
Overall, for small doses, the detriment calculated from the risk estimates for the different potentiel effects on the developing organism appears rather small, in comparison with the natural risks, spontaneously associated with pregnancy.

1. Introduction
It has been known for a long time that the tissues in intense proliferation, that is those containing many dividing cells, are particularly radiosensitive. This is the law formulated as early as in 1906 by Bergonié and Tribondeau, after which the radiosensitivity of a tissue increases as its cells are less differentiated, have a greater potential of proliferation and divide more rapidly. Therefore, it can be

supposed that the embryo should constitute a particularly vulnerable target for radiation, specially during the first steps of development.

Among the somatic effects of radiation other than cancer, developmental effects on the unborn child are of greatest concern. The classic effects are:

1)   Lethal effects induced by relatively small doses before or immediately after implantation of the embryo into the uterine wall or induced after increasingly higher doses during all stages of intrauterine development, to be expressed either before or after birth;

2)   Malformations, characteristic of the period of major organogenesis, when the main body structures are formed, and specially of the most active phase of cell multiplication in the relevant structures;

3)   Growth disturbances without malformations, induced at all stages of development but particularly in the latter part of pregnancy;

4)   Miscellaneous effects on various body structures and functions.

The principal factors of importance are the dose and the stage of gestation at which it is delivered. Dose rate is also of significance, since many pathological effects on the embryo are reduced significantly by reducing the dose rate.

Most experimental data on the effects of radiation in the developing embryo or fetus have been obtained with the mouse or the rat, animals that reproduce in quantity with a relatively short gestation period. The principal events in the development of an embryo, namely, cleavage, implantation, placentation, organogenesis, and differentiation of the various organs, tend to occur in all mammals in roughly the same sequence; it is the time scale that differs. It is probably justified, therefore, to assume that the major effects seen in the mouse or rat when irradiation is delivered at specific stages of development will also occur in humans at the equivalent stages.

Russell and Russell divided the total developmental period *in utero*, corresponding each to a peculiar sensitivity to the various effects of radiation.

-     the first period is the so-called "preimplantation period", and extends from the fertilization up to the implantation of the embryo into the uterine walls. During this, the fertilized egg, or zygote, will travel the oviduct (or Fallopian tube) to arrive after a few days into the maternal uterus. During its journey through the oviduct, the embryo undergoes a series of divisions, reaching the 2-cell, 4-cell, 8-cell and 16-cell stages. The 16-cell embryo is called the "morula", and the 32-cell embryo, ready to implant, is called the "blastocyst".

-     Implantation consists into the attachment of the embryo to the uterus, its penetration through the epithelium and the beginning of the complex interactions of the embryo with its mother. A relatively long period follows then (from the 2nd week up to the 8th one in humans), during which organs are formed according to a well defined sequence for each species. This period is called "organogenesis". Near its end, the human embryo measures about 30 millimeters and

weighs 2-2.7 grammes. It already possesses more than 90 % of the 4.500 structures described for the adult organism.

- The third period is the "foetal period". Like for the organogenesis, the duration of this period shows great variations among species : it lasts nearly 70 % of the total pregnancy in humans, but only 30 % in the small rodents. This is the period of general growth and functional maturation of the newly formed organs. Important developments occur during this period, for example neurogenesis and synaptogenesis, and formation of the external genital organs.

2. Embryonic radiation sensitivity during gestation : results of the animals experiments

2.1. The preimplantation period

In man, it is virtually impossible to investigate events occurring in the embryo before its implantation, because there is no way of knowing whether fertilization took place before the most sensitive radioimmunoassay tests have detected an increased concentration of human chorionic gonadotropin (hCG) in the urines, indicative of trophoblastic activity. However, it is largely admitted that many pregnancies come to an end before having been diagnosed clinically, and even before the first missed menstruation. Direct observations of human preimplantation stages are thus extremely rare and, in order to have an idea of the effects of an exposure to toxic agents like radiation, one is obliged to rely exclusively on results obtained from experiments on laboratory animals.

Letality has been recognized as the main effect of irradiation during the preimplantation period (Figure 1).
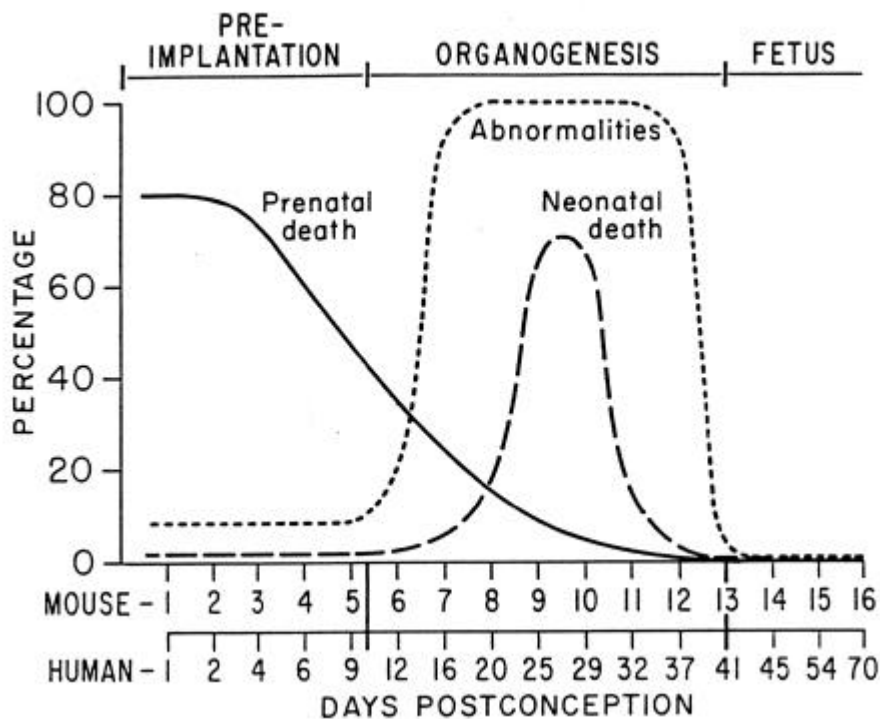
: Incidence of abnormalities and of prenatal and neonatal death in mice given a dose of 2 Gy at various times after fertilization (redrawn by Brent, from L.B. Russell and W.L. Russell : J. Cell Physiol. [Suppl. 1] 43, 103, 1954). The *lower scale* consists of Rugh's estimates of the equivalent stages for the human embryo.

Studies performed with the aid of *in vitro* techniques, and in which we were involved, showed that:

1) Sensitivity to the killing effects of radiation is higher during early stages.
2) For the same embryonic stage, sensitivity can vary by a factor 10 between the different phases of the cell cycle.
3) Sensitivity is highest at the one-cell stage, just after fertilization and before the first DNA synthesis.
4) Embryonic mortality following irradiation at this stage and at later stages occurs predominantly near the time of implantation, and results mainly from structural and numerical chromosome aberrations which develop even several cell cycles after irradiation.

It is generally admitted that early embryos escaping killing by radiation will develop without anomaly, due to the fact that cells are still undifferentiated at these stages and that loss of one or a few cells can be compensated by other cells. This rule suffers, however, at least one exception. Thus, a dose-dependent increase in the frequency of an abdominal malformation, the gastroschisis, was noted after irradiation of mouse one-cell embryos of the Heiligenberger strain with neutrons or X-rays. More details and explanations about these results can be found in the paper by Prof. C. Streffer (next paper in this issue).

In our laboratory, we also performed similar experiments on two other mouse strains, using exactly the same methods. Embryos were also X-irradiated at the one-cell stage, 7 hours after fertilization, corresponding theoretically to the time of highest radiosensitivity during the first cell cycle. We will not enter the details of these experiments. It will be sufficient to say that in the BALB/c strain, there was clearly no increase in the frequency of abnormal fetuses after irradiation, while in the CF1 strain, this frequency showed a tendency to increase, the difference against controls being significant for the doses of 0.5 and 1 Gy.

Irradiation caused no increase of the proportion of fetuses with skeletal anomalies, in agreement with results obtained previously by others.

The apparent increase in the frequency of abnormal fetuses noted in the CF1 strain after X-irradiation is difficult to interpret, because the frequency of abnormal fetuses found in the control group serving for the comparison was exceptionally low. Later on, we performed new experiments in the same strain, and the frequency of abnormal fetuses obtained in the control group appeared much higher, and in fact much less different from those found earlier in the irradiated groups. So, the results obtained in these experiments constitute surely no proof that radiation can be teratogenic in the CF1 mouse strain, when administered during the preimplantation period.

If one examines very critically all the results published in the literature, it appears that the study performed in the Heiligenberger strain is the only one in which a defined dose-dependent increase in the incidence of malformations was noted after irradiation of preimplantation embryos, and it is therefore clear that early mortality will still constitute by far the principal risk of such exposure. It can be estimated that, at the time of highest sensitivity, i.e. the one-cell stage a few hours after fertilization, the mortality would increase of about 1 % per cGy of acute X-irradiation. However, even this risk remains low in comparison with the natural risks spontaneously associated with pregnancy. In this view, it will be useful to precise that 30 to 60 % of human embryos abort spontaneously, while about 2-3 % of the surviving fetuses show a congenital anomaly at term. This rate roughly doubles to 4-6 % if grown children rather than babies are examined.

2.2. The organogenesis period

During this period, the main effect of radiation in small rodents is the production of a variety of congenital anomalies. As seen in figure 1, a dose of 2 Gy to the mouse embryo during the period of maximum sensitivity can result in a 100 % incidence of malformations at birth. A similar result is seen for rats exposed to 1 Gy. For each species, there exists a well determined period of sensitivity to the induction of each malformation. Increasing the dose usually results in an extension of this period of sensitivity and in an increase of the incidence of malformations. The specific time when a malformation is produced coincides with the main stage of differentiation and organization of the considered structure.

It can be admitted that the mechanism causing malformation is the cellular death followed by an arrest of development of the structure at an early stage. By analogy with the observations made on embryos at preimplantation stages or on somatic cells of adult organisms, it seems that cellular death is to be attributed to chromosome aberrations. The apparition of a malformation will depend on the number of damaged cells in the forming organ, and thus on the dose. These effects are typically deterministic. The experimental data show that the form of the dose-effect relationship for the induction of malformations is generally sigmoid, the frequency of malformations by unit of dose increasing with the dose.

Experiments performed in rodents suggested that 10 cGy represent the minimal dose to induce malformations in organs, while some skeletal anomalies could even be induced by 5 cGy.

Embryos exposed during early organogenesis also exhibit the greatest intrauterine growth retardation. This is expressed as a weight reduction at term and is a phenomenon resulting from cell depletion. Animals show a remarkable ability to recover from the growth retardation produced by irradiation during organogenesis, and while they may be smaller than usual at birth, they may achieve a normal weight as adults. There is an association between growth retardation and teratogenesis: irradiated embryos that show major congenital anomalies also suffer an overall reduction of growth. In animals, a dose of about 1 Gy will produce growth retardation when delivered at any stage of gestation (except during preimplantation), while 0.25 Gy does not produce an observable effect even at the most sensitive stage.

If death occurs as a result of irradiation during organogenesis, it is likely to be neonatal death-occurring at about the time of birth. The transition from prenatal death from irradiation during preimplantation to neonatal death resulting from irradiation during organogenesis is very clear in the graph. In this case neonatal deaths peak at 70 % for mice receiving 2 Gy on the tenth day. The deaths probably occur because some grossly abnormal fetuses are unable to develop to term.

2.3. The foetal period

If one excepts the effects on the central nervous system, it can be said that the consequences of an exposure to radiation during the foetal period are much less spectacular. It can induce anomalies in the develement of the tissues, since histogenesis is much active at that time, and general or localized growth retardation. In contrast to what is observed after irradiation during organogenesis, the growth retardation induced during the foetal period frequently persists during all the extra-uterine life. Various other effects have been described in laboratory animals, including effects on the hematopoietic system, liver, and kidney, all occurring, however, after fairly high radiation doses. The effects on the developing gonads have been particularly well documented, both morphologically and functionally. Doses of a few tenths of a gray as a minimum are necessary to produce fertility changes in various animal species.

3. Embryonic radiation sensitivity during pregnancy : experience in humans

3.1. Congenital anomalies

Contrary to what is observed in experimental animals, radiation-induced malformations of body structures other than the central nervous system are uncommon in humans.

According to Brent, this difference could be the result of two factors. First, in rats and mice carefully conducted experiments have been performed, with exact control of dose and precise timing. In humans, by contrast, the limited data that are available have resulted from the random irradiation of relatively few individuals. Second, in humans the sensitive period during organogenesis, when the range of gross anomalies may be produced, represents only one fifteenth of the total period of gestation (3nd and 4th weeks), whereas in small rodents it amounts to one third. On the other hand, development of the central nervous system is taking place for much of the long period of gestation in humans and for this reason is the most likely target for radiation-induced damage.

Following the Tchernobyl accident, several epidemiological studies were conducted in the exposed populations. One of them, subsidized by the IAEA and 6 other agencies of the United Nations, concerned the 825,000 inhabitants of the 3 most affected republics : Ukraine, Belarus and the Russian Federation. During the years following the accident, and in contrast to what had been reported and some medias, no increase of the frequency of congenital anomalies was noted, which could be attributed to the exposure to radiation.

This was still confirmed recently during 2 international conferences, held in 1995 in Geneva and in 1996 in Vienna.

Similar results were found for the children who had been exposed *in utero* during the bombings of Hiroshima and Nagasaki.

3.2. Effects on the central nervous system

However, the exposure of the human conceptus during the last part of pregnancy, the foetal period, may lead to severe mental retardation associated or not with microcephaly, that is a decrease in head diameter . This is a phenomenon which has been carefully studied by the team of Otake and Schull. A child was considered to be affected by severe mental retardation "if he or she was unable to perform simple calculation, to make simple conversation, to care for himself or herself, or if he or she was completely unmanageable or had been institutionalized".

The prevalence of mental retardation in children exposed *in utero* to the atomic bombs in Hiroshima and Nagasaki has been evaluated in reference to gestational age. Almost all of the mentally retarded cases occurred among the individuals exposed in the 8th-25th week post-ovulation, and more specially in the weeks 8 to 15. The period from 8 to 15 weeks corresponds to a rapid increase in the number of neurons and in their migration to the cerebral cortex, where they lose their capacity to divide and become perennial cells. During the period from 16 to 25 weeks, differentiation *in situ* accelerates, synaptogenesis increases and the definitive cytoarchitecture of the brain unfolds.

In 1987, Otake and his colleagues showed that there was some evidence of a threshold in the dose-response for severe mental retardation, although this could not be demonstrated with certitude. In a paper published in December 1996, the team made a reevaluation of the data, based on a larger sample of individuals and added further evidence for a threshold. The 95 % lower limit of the threshold was estimated to be in the range of 0.06-0.31 Gy for the 8-15- week period. For exposure in the 16-25 weeks period after ovulation, the 95 % lower limit of the threshold was 0.28 Gy.

As underlined by the authors, these estimates of risks and the thresholds are associated with a number of uncertainties. The data are limited and the heavily exposed survivors are few. Moreover, some of these individuals had health problems, presumably non-radiation-related, which could account for their mental retardation. There are still other uncertainties associated with these estimates of risk. Errors in the estimation of the tissue-absorbed dose and the prenatal age at exposure could have occurred; and the contribution of other confounding factors in the post-bomb period, including nutrition and disease, are difficult to assess.

Epidemiological studies alone will not be able to solve this question, and experimental studies will remain necessary in order to elucidate the biological processes underlying the severe mental retardation. The morphological features and developmental processes in the histogenesis of the human cerebral cortex are basically the same as in other mammals. It is interesting, therefore, that the period of high susceptibility of the cerebral cortex to developmental damage noted in epidemiological studies of individuals exposed to the A-bomb while *in utero* is consistent with the findings obtained

from exposure experiments in mice. Further systematic studies including animal experiments on the relationship of gestational age to impaired formation of the cranial bones, the areas of the brain involved and the nature of damage could be useful in determining the threshold of the production of radiation effects on the developing central nervous system.

3.3. The problem of cancer

In addition to the effects mentioned above, some studies have established a link between irradiation *in utero* and the subsequent development of leukaemia and other childhood malignancies. The data concerning this type of effect can divided into two groups : those derived from medical exposures and those obtained in survivors of the atomic bombings of Hiroshima and Nagasaki.

The most important epidemiological study performed on children who had been exposed *in utero* for medical reasons is the so-called Oxford Survey of Childhood Cancers. This study was begun in 1955 and its results have been regularly reevaluated. In their last report, the authors have considered all the childhood mortality having occurred before the age of 16, between the years 1953 and 1979 in England, Scotland and the Wales. So, the number of children included in this report is more than 10 times higher than that which had been considered in the first publication of 1958. Stewart and colleagues concluded that children born of mothers irradiated during pregnancy seemed to have an increased risk of leukaemia and solid tumors in their early age. In 1958, 1970 and 1975, the authors estimated that exposure *in utero* to low doses of X-rays increased the relative risk to contract a leukaemia or childhood cancer by a factor 1.4. However, in their last report of 1987, Stewart and colleagues reestimated this risk to 1.94. This constitutes, in fact, a global value for all the period examined. For the years before 1959, the value was generally higher, while it decreased from the years '60. This difference coincided with the improvements of the radiological techniques that had occurred since the end of the years '50, reducing the doses of radiation delivered.

At first, the findings of Stewart were supported by a similar study by MacMahon, performed independently in the United States. But upon extending the study in time and geography, the excess of cancers other than leukaemia disappeared. Other studies at smaller scale also suggested that diagnostic doses of radiation increase the risk of childhood leukaemia, while others arrived to negative conclusions. Because of its importance, it is, however, the Oxford study which received the most attention. That a statistical association exists between an exposure of the foetus *in utero* and the risk to contract some childhood cancers is a fact which is now broadly accepted. But the uncertainty relates to its interpretation : whether it is an indication of carcinogenicity of radiation exposures of the order of 10 mGy or whether it results from some other characteristics of these pregnancies that both make them more likely to be X-rayed and is associated with increased cancer risk in the offspring. In other words, it is still debated whether the X-rays were responsible for producing the malignancies or whether the irradiated mothers constituted a selected group whose children would have shown an increased incidence of neoplasia in any case.

The strongest support for a causal relationship is provided by the twin data. Thus, an important study was performed in Connecticut, on 32,000 twins born between 1930 and 1969. The idea was that

twin pregnancies were principally X-irradiated for indications directly related to twinning (to confirm the twinning pregnancy or to determine the position of the foetus), so that the effect of selection for adverse medical conditions might be expected to be substantially diluted. Thus, one can theoretically exclude a mysterious third factor that could have been responsible for both the X-ray and the cancer. Globally, the results of that study also suggested a causal relationship between exposure and cancer risk. However, the rather limited number of cancers observed limits somewhat the weight of the study.

A larger study was recently performed in all twins born in Sweden between 1936 and 1967, and led to the same conclusions. It indicated that the risk concerned primarily leukaemia and tumors of the CNS. However, again, its authors underlined that the restricted size of the sampling remained a problem.

There are a number of arguments that argue against radiation as the cause of the malignancies. We will only mention the most important of them:

1) An increased sensitivity of the fetus to radiation carcinogenesis has generally not been found in animals where properly controlled experiments can be designed.

2) The observation of a higher relative risk of leukaemia for exposure during the first trimester of pregnancy than during the last two is implausible on embryological and radiobiological grounds, since its is known that at that time adult hematopoiesis has not yet begun and target cells to be transformed into leukaemic cells have not presumably differentiated.

3) The fact that in the prenatally exposed infants the leukaemias and all the major groups of solid tumors appear to be increased almost equally in relative terms appears to be in contrast with the well known variability of different tumour histotypes to the carcinogenic action of radiation.

4) The results of Stewart and colleagues suggest that the frequency of cancers in the areas of relatively high levels of natural radioactivity should be higher than normal too. Such increase has not yet been observed.

5) *In utero* exposures to the atomic bombings in Japan (mean uterine dose : 0.18 Gy) were not linked to an increase in childhood cancer. During the first 14 years of life of the *in utero* group, no leukaemias occurred and only two cancers were diagnosed: one case of liver cancer at age 6 years and one case of Wilm's tumour at age 14 years. The sample, however, is not large.

To conclude, studies of *in utero* exposure have given a wide range of risk estimates from high to none at all. Despite the many incertitudes that remain, and on account of prudence, the scientific international committees like UNSCEAR, ICRP and BEIR decided to admit the existence of a risk of childhood cancer resulting from an *in utero* irradiation, with a constancy of the risk during all pregnancy. The excess absolute risk of mortality of all cancers for the first 14 years could vary from 200 to $500/10^4$ person Gy.

A distinction must still be made between cancers occurring during childhood and those occurring later, in the adult life. The last results published by Yoshimoto and colleagues indicate an increase of

the incidence of cancers near the beginning of the dult age, in people having been exposed *in utero* to the atomic bombings. However, these studies are far from being terminated and, as underlined by Yoshimoto himself, suffer an important degree of uncertainty, due to the reduced size of the examined cohort.

4. Conclusion and risk estimates

Table 1 summarizes the different potential effects resulting from an *in utero* exposure to ionizing radiation. The values are those admitted by the most important scientific committees, on the basis of the experiments performed in animals and the observations realized in man.

Table1 : Risk estimates in humans for various effects induced by irradiation *in utero*

Effect period in weeks	Relative length of the effect period over pregnancy	Effect	Risk estimate per 10 mGy	Spontaneous frequency (excluding any exposure to radiation)
0-2	0.05	Prenatal death	0.01[a]	0.30-0.60
3-8	0.6	Malformation	0.05[b]	0.06
8-15	0.21	Mental retardation	0.004	0.005
16-25	0.26		0.001	
0-38	1.00	Leukaemia or other childhood cancer	0.0002-0.0005[c]	0.0005

a: estimation for the most sensitive stage to such effect, i.e. the first 10 h following fertilization. Thereafter, the sensitivity greatly decreases.

b: from a threshold dose of 100 mGy for the various organs and of 50 mGy for some skeletal anomalies (in the mouse).

c: i.e. 2-5 %/Gy.

The values given in Table 1 should be viewed in the light of a number of remarks:

Firstly, the risk estimates have been derived under the assumption of linearity, although many dose-effects relationships for the non-stochastic effects, have actually been shown to be curvilinear (or concave upwards). They are therefore likely to be in excess by some unknown factor, particularly at the very low doses.

Secondly, the estimates have been derived by data on acute irradiation at high dose rates, and it is probable that projection of these estimates to low dose-rate low LET irradiation would cause these estimates to drop by some factor.

Thirdly, on account of prudence, the committees admit the possibility of teratogenic effects in man, at the smallest doses able to induce such effects in animals, although, in man, those have been observed only after exposure to very high doses.

Finally, all these risk estimates must, of course, be weighted according to the effective period for the relevant effects, in order to obtain relative estimates applying over the whole period of pregnancy.

Overall, for small doses, the detriment calculated from the risk estimates given in this table appears rather small, in comparison with the natural prevalence of malformations at birth or after a few years, which is about 6 %. For example, an acute dose to the fetus of 10 mGy would add a probability of health effects of all kinds of the order of 0.2 or 0.3 % to the natural probability of 0.06 of a newborn child being malformed.

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Résumé

Le développement embryonnaire peut être grossièrement divisé en trois périodes: (1) la période pré-implantatoire, qui s'étend de la fécondation de l'embryon jusqu'à son implantation dans l'utérus maternel; (2) la période de l'organogenèse, durant laquelle les différents organes sont formés; (3) la période foetale, correspondant à la croissance des organes nouvellement formés chez un organisme qui possède déjà toutes les caractéristiques morphologiques de l'espèce. Les expériences réalisées chez l'animal de laboratoire ont montré que chacune de ces périodes est caractérisée par une sensibilité particulière aux radiations ionisantes et aux autres agents toxiques. Ainsi, il est largement admis que le principal risque d'une irradiation durant la période pré-implantatoire est la mort de l'embryon. Une irradiation durant la période de l'organogenèse pourra caractéristiquement entraîner la production de différentes anomalies congénitales. L'induction de ces anomalies dépendra du nombre de cellules lésées dans l'organe en formation et ne sera observée qu'à partir d'une certaine dose-seuil (50 mGy chez la souris). L'irradiation durant la période foetale pourra induire des anomalies tisssulaires et un retard de croissance général ou localisé. Le retard de croissance induit durant cette période persiste fréquemment durant toute le vie extra-embryonnaire.

Chez l'homme, des malformations radio-induites autres que celles touchant le système nerveux central sont rarement observées. Toutefois, l'exposition de l'embryon humain au cours de la dernière partie de la grossesse, la période foetale, peu conduire à un retard mental sévère associé ou non à de la microcéphalie (diminution du diamètre de la tête). Les données obtenues chez des enfants qui avaient été exposés *in utero* aux bombes atomiques d'Hiroshima et Nagasaki ont montré que la probabilité d'un RMS (retard mental sévère) était essentiellement nulle pour une exposition survenant avant la huitième semaine de la grossesse, était maximale pour une exposition survenant entre les huitième et quinzième semaines et diminuait ensuite fortement lorsque l'exposition avait lieu entre les quinzième et vingt-cinquième semaines. Outre ces effets, certaines études ont établi un lien entre une irradiation *in utero* et le développement subséquent de leucémie et d'autres tumeurs infantiles. Bien que loin d'être prouvé, ce risque est à présent accepté par prudence par les comités scientifiques les plus importants.

Globalement, et pour de petites doses, les risques potentiels d'une exposition prénatale aux rayonnements ionisants apparaissent faibles, surtout en regard des risques spontanés qui accompagnent la grossesse.

Samenvatting

De embryonale ontwikkeling kan in drie perioden worden ingedeeld: (1) de pre-inplantatieperiode die de periode bestrijkt tussen de bevruchting en de aanhechting van het embryo aan de uteruswand; (2) de organogenese tijdens dewelke de voornaamste organen tot ontwikkeling komen; (3) de foetale periode waarin de nieuw gevormde organen uitgroeien. Experimenten die werden uitgevoerd in laboratoriumdieren hebben aangetoond dat elk van deze perioden gekenmerkt wordt door een specifieke gevoeligheid t.o.v. ioniserende stralen en andere toxische agentia. Aldus wordt algemeen aangenomen dat bestraling tijdens de pre-implantatieperiode voornamelijk tot embryonale sterfte leidt, terwijl verscheidene soorten congenitale afwijkingen het kenmerk zijn van een bestraling tijdens de organogenese. Het optreden van anomalieën hangt af van het aantal beschadigde cellen in het betrokken zich ontwikkelend orgaan. Afwijkingen zullen niet voorkomen beneden een zekere drempelwaarde (50 mGy in de muis). Bestraling tijdens de foetale periode kan afwijkingen induceren in de ontwikkeling van weefsels, zowel als algemene of lokale groeiachterstand. Groeiachterstand die in deze periode is opgetreden zal vaak blijven bestaan tijdens het extra-uterine leven.

Bij de mens komen andere dan stralingsgeïnduceerde misvormingen aan het centraal zenuwstelsel zo goed als niet voor. Toch zal stralingsblootstelling tijdens de foetale periode kunnen leiden tot ernstige mentale achterstand en dit eventueel in associatie met microcefalie (verminderde diameter van het hoofd). Gegevens bekomen bij in utero blootgestelde kinderen als gevolg van de A-bom explosies in Hiroshima en Nagasaki hebben aan het licht gebracht dat de kans op ernstige mentale achterstand zo goed als onbestaande is wanneer het blootestelde embryo minder dan 8 weken oud is. Het risico op ernstige mentale achterstand wordt echter maximaal wanneer het embryo tussen 8 en 15 weken oud is. Embryo's die tussen 16 à 25 weken oud zijn zien het risico dan weer verminderen.

Enkele studies hebben daarenboven ook een verband gelegd tussen de bestraling in utero en het ontstaan van leukemie en andere kankers bij kinderen. Alhoewel dit zeker nog niet is bewezen aanvaarden de belangrijkste wetenschappelijke instanties tegenwoordig toch het bestaan van dit risico op basis van het voorzorgsprincipe.

Algemeen genomen tonen risicoschattingen voor de verschillende potentiele effecten op het zich ontwikkelend organisme aan dat het risico eerder gering is in vergelijking met de natuurlijke risico's die met een zwangerschap gepaard gaan.

GENETIC PREDISPOSITION AND GENOME INSTABILITY AFTER IRRADIATION IN MOUSE PREIMPLANTATION EMBRYOS.

C. Streffer

Institut für Med. Strahlenbiologie,
Universitätsklinikum Essen,
45122 Essen, Germany

Abstract:

The preimplantation mouse embryo is a useful system for radiobiological studies. Chromosomal aberrations were determined after exposure to X-rays and neutrons during the zygote (1-cell stage). New aberrations developed and were expressed during the 2nd and 3rd mitosis after irradiation. These later aberration developed from DNA damage which was originally not a double strand break (DSB). Further chromosomal aberrations were studied in fibroblasts of fetuses 19 days post conception. A significant increase of chromosome aberrations was found in the fetuses which were irradiated in the 1-cell stage and which had developed a malformation. These data can only be explained by the induction of a genome instability through the radiation exposure which had been performed many cell generations earlier.

Until recently it was generally accepted that an exposure to ionizing radiation during the preimplantation period of mammalian development will not induce malformations. However, recently it could be shown that certain sensitive mouse strains exist in which malformations are induced by exposure to X-rays and neutrons during the preimplantation period. It was further demonstrated that this effect can be suppressed if the sensitive mouse strain is crossbred with mice from a resistant mouse strain. These data show that this radiation effect is due to a genetic phenomenon with a recessive trait. Studies on protein patterns in normal fetuses and in fetuses with the malformation showed that characteristic changes occur. There are proteins which are no longer expressed in the malformed fetuses and new proteins may appear. Certain changes in glycoproteins and phosphoproteins were

found not only in liver of malformed fetuses but also in skin and kidney of these organisms. The analysis of the genome of these malformed fetuses have given evidence that changes in two or three genes are responsible for the radiation-induced malformation. It was possible to localize 1 gene on chromosome 7 with high gene linkage in a region in which several genes are located for imprinting processes. These malformations which are induced during the preimplantation period occur in those mouse strain which have a genetic predisposition.

1. INTRODUCTION

The development of the preimplantation mouse embryo can be followed in vitro by incubating the embryos in comparatively simple media [1]. By this techniques the development can be followed from the 1-cell embryo (zygote) to the blastocyst and the hatching of the blastocysts. It has been demonstrated that this system has great advantages for radiobiological studies [2]:

1. It can be followed easily under the microscope how many mitotic cell divisions have taken place after conception.
2. The cell cycle phases are very well defined, the duration of the various cell cycle phases has been determined exactly. Especially during the first three cell cycles the synchrony of the cells within individual embryos is comparatively high and therefore it is always well-known at which phase a treatment (cf exposure to ionizing radiation) has taken place.
3. All cells participate in the cell proliferation. Therefore the growth fraction is 1.0.
4. The development of the embryos in the in vitro culture is very similar as the in vivo development. It is possible to test the quality of the embryo by transplanting the embryo into fostered mice and observing the development in vivo.

5. The development of the preimplantation embryos of mice is very similar as in humans, therefore the extrapolation from mice to humans is easily possible.

A disadvantage of the system may be the specific processes which are directly connected to the development of the embryo like specific protein expression and therefore there are some differences from cell cycle to cell cycle.

2. DEVELOPMENT OF CYTOGENETIC DAMAGE

For the studies of chromosomal aberrations the preimplantation mouse embryos were irradiated during the first cell cycle (1-cell stage; zygote) at various times post conception (p.c.). It could be demonstrated that the frequency of chromosomal aberrations measured in the first mitosis post radiation varied appreciably in dependance on the radiation time. This means that the radio sensitivity is very different at various stages of this cell cycle [1, 2]. In further experiments the number of chromosomal aberrations was measured at the first, second or third mitosis after irradiation with X-rays or neutrons [2, 3]. The radiation exposure took place 1 h p.c. in most cases. After X-ray exposure chromosome breaks were higher than chromatid breaks at the first mitosis. However, in the second and third mitosis the frequency of chromatid breaks was higher than chromosome breaks and there was an appreciable expression of new chromosomal aberrations in these later mitotic divisions. Very similar situations were observed after neutron irradiation although the ratio of chromosome breaks and chromatid breaks was somewhat different in the second mitosis [3, 4] (Table 1).

Table 1: Chromosomal aberrations per metaphase in mouse preimplantation embryos after exposure to 0.94 Gy X-rays or 0.37 Gy neutrons 1 h p.c. [10].

	X-rays		Neutrons	
	Chromos. br.	Chromat. br.	Chromos. br.	Chromat.br.
1st mitosis p.r.	0.150	0.017	0.286	0.043
2nd mitosis p.r.	0.033	0.098	0.092	0.092
3rd mitosis p.r.	0.057	0.100	0.091	0.204

It is generally assumed that chromosome breaks and chromatid breaks are the consequence of DNA double strand breaks (DSBs). If this is also the case for the chromosomal aberrations which are expressed during the second and third mitosis post radiation it follows that the DSBs must have developed by processing some DNA damage into DSBs during the second and third cell cycle after irradiation. This means that the primary damage was apparently not a DSB in these cases but these breaks have developed later. This assumption could be confirmed by

experiments which have been performed with restriction enzymes. The preimplantation mouse embryos were incubated with the restriction enzyme Alu I. Under these conditions no increase of chromosomal aberrations was observed in the third mitosis [5].

3. INDUCTION OF MALFORMATIONS

It is generally assumed like a dogma that ionizing radiations do not induce malformations in mammals when the exposure takes place during the preimplantation period [6]. In our laboratory we are working with a mouse strain (Heiligenberger mice HLG) which has a comparatively high spontaneous rate (1-3 percent) of a special malformation (gastroschisis). In this mouse strain it was observed by us that a radiation exposure during the zygote stage to X-rays or neutrons increased the number of gastroschisis in a dose dependent manner [7, 8]. As expected neutrons were more effective by a factor of about 2 to 3. For comparison experiments were performed under the same conditions with a second mouse strain (C57 BI). In this mouse strain it was not possible to induce malformations by ionizing radiation exposure during the preimplantation period. Further the rate of malformations was reduced when a cross breeding between the 2 mouse strains was performed, the irradiation took place again at the zygote stage and the malformations were studied in the resulting F1-generation [9]. Very similar data were obtained by Generoso et al. [10] when preimplantation mouse embryos were exposed to the alkylating agent ENU in utero.

Table 2:   Percentage of fetuses with skeletal malformations (observed on day 19 p.c.) in the group of fetuses with or without gastroschisis (Number of malformed/ total fetuses).

	Without	With
	Gastroschisis	
Control	0.6% (5/891)	9.1%(3/33)
Irradiated stage: preimplant. (day 1 or 2 p.c.)	1.4% (8/570)	11.7% (7/60)
Irradiated stage: postimplant. (day 6, 7 or 8 p.c.)	17.2% (75/436)	28.3% (15/53)

In the HLG-strain not only gastroschisis but also other skeletal malformations were observed however in a much lower frequency after irradiation during the preimplantation period. It was found that fetuses with a gastroschisis had a much higher risk for other skeletal malformations than fetuses with no gastroschisis [9] (Table 2). From these data it was concluded that apparently not only a genetic predisposition exists in the HLG mice which leads to the induction of gastroschisis even after an irradiation during the preimplantation period but also a genome instability was induced which leads to further malformations.

In order to verify the genetic predisposition, protein patterns were studied in normal fetuses and in malformed fetuses. It was found that in the pattern of liver proteins of malformed fetuses some new proteins occurred, some proteins were lost and structural changes had occurred in other proteins. These results were obtained by two-dimensional-gel-electrophoresis and densitometric measurements of the protein spots in electropherograms [11]. Further it was found in homogenates of fetal liver, kidney and skin by P-32-phosphate labelling that a certain phosphorylated cytokeratin was reduced in the malformed fetuses in all 3 tissues [12] (Table 3). Of special interest in this connection is also the reduced intensity of glycoproteins in the skin of malformed fetuses. These data especially the occurring changes of the same kind in three different tissues underline the assumption that the radiation induced effect of malformations is based on genetic radiation damage.

Table 3:   Integrated intensities of a phosphorylated cytokeratin in control fetuses (n = 10) and fetuses with gastroschisis after X-irradiation with 1 Gy 3 h p.c. [19]

Tissue	Control	Gastrosch.	P
Liver	10.1 ± 1.8	3.5 ±0.2	<0.001
Kidney	2.8 ±0.4	1.9 ±0.1	<0.05
Skin	5.6 ±0.6	4.2 ±0.1	<0.05

With respect to the dose effect curve it was of further interest that the dose effect curve for radiation induced gastroschisis had no threshold dose when the irradiation took place during the 1-cell stage (zygote). However, a threshold dose was observed when the irradiation was performed with multicellular embryos [13].

This underlines the assumption that a dose effect curve without a threshold will only be observed when the radiation effect will develop from an unicellular event.

From the data which had been obtained thus far it could be concluded that the damage had a genetic basis with a recessive trait. The latter is concluded from the data after crossbreeding. Further the genes which are responsible for this effect are apparently more radiosensitive than others so that the radiation effect can be seen to that extent after irradiation during the preimplantation period. From other experimental data it can be concluded that the expressed radiation damage is not randomly distributed over the whole genome but that certain genes or locations in the genome are more radiosensitive than others. This is certainly in contrast to earlier assumptions. Therefore experiments were undertaken in order to characterize the genes which are responsible for the induction of gastroschisis. First it was tried to find out how many genes are involved in this process. For these experiments HLG mice were crossbred with C57 BI mice and the obtained female F1 generation was mated with male HLG-mice (back cross, BC1). The females were irradiated after mating when the embryos were still in the zygote stage. From the frequency of malformations in the original HLG mice and in C57 BI mice, in the F1 generation and in the backcross (BC1) it could be analyzed that 2 to 3 genes are involved in the formation of gastroschisis [14]. In further experiments we have started to perform a genome-wide search for the location of these genes by DNA-hybridization techniques with microsatellite markers. The microsatellite typing has resulted that 1 of the genes is located on chromosome 7 in a region where several genes for imprinting processes are located [15].

4. GENOME INSTABILITY

The data on the induction of gastroschisis and the genetic predisposition show already that there is some genome instability in the HLG-mouse strain which is increased by ionizing radiation. This is underlined by the result that fetuses with gastroschisis have also an increased rate of skeletal malformation. Such a genome instability could also be shown by the study of chromosomal aberrations in fetal fibroblasts. For these experiments the zygotes were irradiated with X-rays and fibroblasts were obtained from fetuses with gastroschisis or without gastroschisis on day 19 p.c. just before birth. The fibroblasts were brought into cell culture and 48 hours later the number of chromosomal aberrations was studied. It turned out

that Xrays induced a higher frequency of chromosomal aberrations in the fetal cells which were obtained many cell generations after the radiation exposure had taken place. This effect was especially pronounced in those fetal cells which had been obtained from fetuses with gastroschisis [16] (Table 4). These data clearly show that genomic instability is induced by X-ray exposure and it is expressed many cell generations later. Such a genome instability has a great significance also for other radiation effects as carcinogenesis where several mutation steps are involved.

In further experiments it could be demonstrated that the radiation induced genome instability is transmitted to the next generation [17].

Table 4:   Number of chromosomal aberrations per 100 metaphases in fibroblasts from fetuses without irradiation (Controls) (19 d p.c.) and fetuses without (Normal) and with gastroschisis (Gastrosch.) after X-irradiation with 2.0 Gy (1 h p.c.) [16].

	Studied metaph.	No. of aberr. per 100 metaph.
Contr.	400(8)	8.25
Norm. p.r.	322(8)	20.50
Gastrosch. p.r.	503(10)	25.05

CONCLUSIONS

1. The preimplantation mouse embryo is an excellent model for radiobiological studies due to cell synchrony, rapid cell proliferation and easy observation of cell division.
2. Chromosome aberrations are expressed during the first mitosis post irradiation but further aberrations are observed during the second and third mitosis.
3. After neutron exposure chromosome breaks dominate in the first mitosis but chromatid breaks in the third mitosis.
4. Radiation damage of the DNA is processed during the post radiation cell cycles and expressed as aberrations at later mitosis.
5. Malformations are induced by exposure to ionizing radiation during the preimplantation period.
6. This effect can be observed only in certain mouse strains with a genetic predisposition and some genome instability. The expression of proteins is modified in fetuses with radiation induced malformations.
7. The effect is caused by multifactorial genetic changes which have broad implications for population genetics.
8. In fibroblasts of fetuses which were irradiated in the zygote stage an increased number of chromosome aberrations is observed many cell generations after the X-ray exposure as a sign of genome instability. This effect is very significant for cancer induction.
9. This genome instability is transmitted to the next mouse generation.

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Résumé

L'embryon pré-implanté constitue un système intéressant pour les recherches en radiobiologie. L'induction d'aberrations chromosomiques a été étudiée chez des embryons qui avaient été exposés à des rayons x et à des neutrons durant le stade du "zygote" (stade 1-cellule). De nouvelles aberrations se sont développées et exprimées au cours des deuxième et troisième mitoses suivant l'irradiation. Ces aberrations tardives se sont développées à partir de dommages qui, à l'origine, n'étaient pas des cassures double brin (DSB ou "double strand break"). Les aberrations chromosomiques ont encore été étudiées à un stade plus tardif, dans les fibroblastes de foetus au 19e jour de la gestation. Un accroissement significatif du taux d'aberrations chromosomiques a été constaté chez les foetus qui avaient été irradiés au stade 1-cellule et qui avaient développé une malformation. Ces données ne peuvent s'expliquer que par l'induction d'une instabilité génomique par l'irradiation délivrée un grand nombre de générations cellulaires auparavant. Jusqu'à présent, il était généralement admis que l'exposition aux radiations ionisantes durant la période pré-implantatoire du développement embryonnaire ne pouvait induire de malformations. Cependant, on a récemment découvert qu'il existait certaines races de souris sensibles chez qui des malformations pouvaient être induites par une exposition aux rayons x et aux neutrons durant cette période. On a aussi démontré que cet effet pouvait disparaître si les souris de la race sensible étaient croisées avec des souris d'une race résistante. Ces données montrent que cet effet des radiations est dû à un phénomène génétique à caractère récessif. L'étude comparative des protéines synthétisées chez des foetus normaux ou affectés de la malformation a montré des différences caractéristiques entre ceux-ci. Ainsi, certaines protéines n'étaient plus exprimées chez les fœtus malformés, alors que de nouvelles autres apparaissaient. Certaines modifications de glycoprotéines et de phosphoprotéines ont été trouvées non seulement dans le foie des foetus malformés, mais aussi dans leur peau et leurs reins. L'analyse du génome de ces foetus malformés a suggéré que des modifications de deux ou trois gènes seraient responsables des malformations radio-induites. Il a été possible de localiser 1 gène sur le chromosome 7, possédant un linkage génique élevé dans une région possédant plusieurs gènes impliqués dans des processus d'empreinte génomique. Les malformations radio-induites durant la période pré-implantatoire ne surviennent que chez des races de souris ayant une prédisposition génétique à ce type d'effet.

Samenvatting

Het preimplantatie muis embryo systeem is een zeer belangrijk biologisch systeem voor studies in de radiobiologie. Chromosomaleafwijkingen werden vastgesteld na blootstelling van de zygote (1-cellig stadium) aan X-stralen en neutronen. Nieuwe afwijkingen ontstonden en kwamen tot uiting gedurende de 2e en 3e deling na bestraling. Deze late aberraties ontstonden vanaf DNA schade die oorspronkelijk niet met een dubbel strand DNA breuk (DSB) overeenkwam. Verdere chromosomale afwijkingen werden 19 dagen na de conceptie bestudeerd in fibroblasten van foetussen. Er werd een statistisch significante toename van afwijkingen waargenomen in foetussen die werden bestraald in het 1-cellig embryonaal stadium en die een misvorming ontwikkelden. Deze gegevens kunnen alleen verklaard worden door de inductie van genoominstabiliteit gedurende de blootstelling die vele celgeneraties eerder optrad. Tot voor kort werd algemeen aangenomen dat een blootstelling aan ioniserende stralen gedurende de preimplantatie periode in zoogdieren geen misvormingen induceert. Recent werd echter het bestaan van bepaalde gevoelige muisstammen aangetoond waarin blootstelling aan X-stralen en neutronen tot misvormingen leidt. Dit effect kon bovendien worden voorkomen via kruising van deze dieren met een resistente soort. Dit betekent derhalve dat het stralingseffect het gevolg is van een recessief genetisch fenomeen. Studies van proteïnepatronen in normale en misvormde foetussen toonden ook karakteristieke verschillen aan.

In misvormde foetussen werden nieuwe proteïnen aangetroffen terwijl andere proteïnen niet meer tot uitdrukking kwamen. Er werden veranderingen aangetoond in glycoproteïnen en fosfoproteïnen in de lever van misvormde foetussen, maar ook in de huid en nieren van deze organismen. Analyse van het genoom van deze misvormde foetussen heeft gegevens aangereikt waaruit blijkt dat veranderingen in twee of drie genen voor de stralingsgeïnduceerde misvormingen verantwoordelijk zijn. Het was mogelijk 1 gen met hoge 'gene linkage' op chromosoom 7 te lokaliseren en wel in een gebied waar verschillende genen voor 'imprinting processen' gelokaliseerd zijn. Deze misvormingen die gedurende de preimplantatieperiode worden geïnduceerd komen voor in deze muisstammen die een genetische predispositie vertonen.

THE ETHICAL ASPECTS OF PRENATAL IRRADIATION

Dr. P. Smeesters
Ministry of Social Affairs, Public Health and Environment
Radiation Protection Department, Brussels

The basis of ethical judgment

The absence of philosophical consensus

Today, the ethical preoccupation has loosed itself from the religious or ideological context in which it was deeply rooted . As Jean LADRIERE said, it has grown into a "relatively autonomous field of Culture".
Nevertheless, there are meeting points between the various religious traditions which could facilitate an ethical consensus. Hans KÜNG draws a parallel between commonly shared modern values, on the one hand, as concern for peace, solidarity, concern for truth and transparency and equality between men and women, and four classical Commandments of many religions, on the other hand : thou shalt not kill, thou shalt not steal, thou shalt not lie, thou shalt not commit adultery…
Whatever it may be, the aim of ethical life has been "thought" by nearly all philosophical traditions as the quest for *Good*. Paul RICOEUR adds: " quest for a good life, *with and for others*".
This being said, one has to recognize that , in many areas and in particular on questions regarding the protection of the embryo, there is no consensus on the philosophical basis upon which to stand , which practically implies the development of strategies to obtain a consensus *for a specific decision in a specific situation.*

The ethical judgment is situation-oriented

This direct relation with action and situation is a typical feature of the ethical approach and finds a good application in the area of prenatal irradiation.
There are issues facing legislators – or their advisors – who, as representatives of the public interest in a democratic society, have to draw up general radiation protection standards to be followed when carrying out "practices" or preparing "interventions".

A different type of reasoning is required for the management of the consequences of an accidental irradiation of a pregnant woman, whether she be an occupationally exposed worker or a patient whose pregnancy was unsuspected. In this case, it is not a question of drawing up ethically-justified protection standards for future exposure which, in theory, can be limited or avoided, because in this instance the exposure has already occured, the damage has already been done and we have to deal with the consequences.
 Specific approaches are also required in the frame of medical exposures, in particular for exposure of female patients as part of their own medical diagnosis or of a woman knowingly and willingly helping in the support of a patient – for example her child -,

after a treatment with radionuclides. Here the doses are not yet received and we are in a process of balance between various risks and/or detriments: risk for the unborn child, medical benefit for the mother, psychological issues, …

Ethical judgment implies first a cognitive interpretation

In many current debates, ethical judgment has to be elaborated on very technical matters and supposes a good comprehension of the scientific background. Ladrière says that "the ethical judgment implies first a *cognitive judgment*". Unfortunately, in most cases, there are important residual *uncertainties* and there remains a lot of interpretative work to be done, which may lead to varying recommendations or decisions.

The reasons for " reading" a reality in a different way may result from the *mandate* of the experts. Whether we want it or not, everyone of us, including experts, addresses the public with a different mandate, the nature of which influences the primary goals which are aimed at. This can obviously affect the expected degree of neutrality.

There are also many interferences of *ethical* judgments *in the process itself* of scientific evaluation, as we will see later.

But when I presume I am free of all obligations, of all mandates, and if I
limit myself to my scientific field, i.e. the observation of reality – a photography of reality, said Claude BERNARD – would it not be possible for me, then, to come to a one and only truth in this particular field? This is not sure…modern reflection about science has clearly shown that the role of the subject who observes (as an isolated individual or, more often, as a representative of a current of thoughts) is essential, whether this is in the process of selecting observations, or in the formulation of hypotheses or in the theoretical construction.
To take up again the photographic analogy, we are not all cast in the same mould : we do not make a photograph, we can only *interpret.*

Uncertainties and interpretations in the field of the risks of prenatal irradiation

Preimplantation period

Although many international organizations, including ICRP, state that lethal effects at this stage don't appear below 100 mGy, others, like consultants for the NEA, mention in their report a possible exception in the case of certain animal strains with a particular genetic sensitivity, where the inactivation threshold may be as low as 50 mGy. What do we know about the threshold, or variations in threshold, in human beings? What about the possible role of genetic predisposition?
Furthermore, are we really in a position to assert that effects during this stage are always of the all-or-nothing type and can we exclude, after the experiments of STREFFER and others, the possibility of teratogenic effects of a stochastic kind, either in all people, or in sensitive subpopulations ?

Organogenesis stage

The same is true with regard to the level of the dose thresholds. It is currently accepted that a value of 100 mGy may be used as a threshold for recognisable major malformations. Here again, the NEA's report, mentioned above, quotes a dose threshold for radiation-induced effects of 50 mGy during the induction period at the beginning of the organogenesis stage, where severe and often lethal radiation-induced lesions occur. They also point out that the degree of intra-uterine selection, and therefore the tendency to abort,   may vary according to the population studied.
A recent NCRP document also mentions a possible- but serious !- exception to the 100 mGy threshold value: the possible radiation induction of small head size for doses as low as 50 mSv.
Can we say that we have enough data available to us to draw a definite conclusion for the human embryo?

The period of development of the Central Nervous System

The observed data (during the critical period from the 8th to the 15th week) have a stochastic side, in that sense that the probability of their apparition increases with the dose (with a dose/effect relationship statistically compatible with a linear response) and that they already appear in the case of relatively low doses, without a statistically demonstrable dose threshold.

The situation clearly has a stochastic side, but the mechanisms generally put forward in order to explain it, are of a determinist nature (dose-linked decrease of IQ). The explanation assuming a deterministic mechanism with a shift of the Gaussian IQ curve, actually implies an increased probability of severe mental retardation in line with dose together with an apparent absence of a threshold, in the sense that only IQ borderline individuals may be pushed over the borderline and suffer effects that are clinically more pronounced .

This explanatory hypothesis supposes a classical mechanism based on a direct relationship between the severity of an effect and the number of cells affected; it implies that a potentially intelligent human being will not become retarded after having received a low dose and that only those who are less intelligent to begin with are at risk (this does not mean that it is less important).

On the other hand, the biological effects involved are probably not limited to cell death, as in the classic deterministic mechanisms, but also include failures in migration or errors in synaptogenesis. The work of RAKIC and other researchers indicate that the cortex might be a collection of developmental columns each arising from a specific proliferative unit. In this framework, the loss of a few cells could result in the loss or deterioration of specific brain functions.

This raises an epistemological question. Are the conclusions drawn by some from the Japanese data not based on a false premise, i.e. that the radiation induced mechanisms are either stochastic or deterministic.

Reality may sometimes be more complex and, in this case, there may be a combination of deterministic effects based on the quantity of the cells affected, and of random effects based on the quality of the cells affected or of the mechanisms disturbed..

Whatever the solution of this problem might be , I would like to emphasize the following point : identical facts can lead to various interpretations which are equally scientific, even if they are not classic. The weight of the theoretical classic distinction between long-term stochastic effects through mutation, and non-stochastic effects through cellular inactivation, and the weight of the scientific consensus on this should not result in a sort of anathema denying the existence of other interpretations of the observed data.

Prenatal irradiation: ethical questions in the process of scientific evaluation

Potential implications of the expert's mandate

We have seen that anyone of us, including experts, addresses the public with a different mandate, the nature of which may influence the primary goals that are aimed at.
There is nevertheless a demand –sometimes implicit- for recognition- recognized character of the competence-, objectivity of the advice given and neutrality . The "civil" society is more conscious of the ambiguity in the relationships between science and power , which explains the demand for experts who are credible in the eyes of all parties.
This must be of concern to the experts who have to set high standards for their own deontology, to claim, on ethical bases, a freedom of speech or, at least, to be perfectly clear regarding the possible limitations inherent to their mandate.
 The transparency at this level is also a prerequisite for credibility.

Risk of confusion between scientific evaluation and ethical judgment

Some of you perhaps remember this old medieval image regarding the three ways of entering the human knowledge: "the eye of flesh, the eye of reason and the eye of contemplation".

This way of looking at things has not yet lost touch of reality. The eye of flesh allows us to observe immediately what can be observed, measured, quantified; the eye of reason takes over when the eye of flesh has gone blind, its domain being that of reasoning, of logic, of theoretical constructions; and we will always arrive at a point where man is confronted with mystery : this is the domain of human rights, of ethics, the domain of the sacred for the believers, of "essential" values for the agnostics : the eye of contemplation.
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Such ethical issues exist in the area of scientific evaluation and can be sources of varying opinions, including from"neutral and objective" experts, opinions that have to be accepted and respected.
Here are some examples.

When the facts and their limitations are identified, how then do we have to react facing the residual *uncertainties*; should we not opt for being careful, and next to that, how far do we have to go ? Let us remember, regarding this, the issue of setting *definite* dose thresholds for deterministic effects in an embryo.

But, … Is the embryo a person ?At what age does it become one ? At three months ? On the point of being born ? Has it any rights ? Has it the right to be protected ? Does it have to be protected as a member of the public involuntarily exposed to ionizing radiations ? Or, should it be protected even better, because it runs a greater risk, specifically at the level of cerebral neurons ?
In particular, is the death of a very young embryo – or a zygote-, of no importance, as suggested by some international recommandations, due to the fact that the early embryonic lethality is normally high and that this death would be undetectable by the woman? Actually, the evaluation of such risks should also include what is called its "existential significance" (P.P. DRUET et al).

Problems arise when the *specificities* of the above mentioned various ways to knowledge are *denied a*nd when we fail to limit ourselves to our specific field of knowledge.
With regard to the scientist, nowadays at the top of social prestige, he has to fight against this natural tendency of leaving his domain and of *using his authority* in certain areas in which he is no more competent than other people, such as ethical ones.
Nevertheless, in order to allow for a debate, the expert has a specific responsibility, namely that of making clear, in all honesty, what the remaining uncertainties are and what is at stake. He also has to translate the terms of this issue into a language accessible to the actors of the debate. This is a far more difficult exercise than it may seem.

Management of masses of scientific sources

Even the most honest and the most intelligent scientist in unable to read and evaluate all that is published, particularly when he has to cope with broad fields of investigation. Adequate strategies to take up the challenge are currently tested at the level of the European expert groups in the field of the Basic Safety Standards: during regularly organised seminars, high-level specialists involved in European research try to synthetize the state of the art in a particular domain and the potential implications, together with regulators and peer reviewers chosen by the Member States' experts.

Prenatal irradiation: ethical questions in the decision- making process

Determination of standards

This section deals with the issues facing legislators – or their advisors – who have to draw up general radiation protection standards to be respected when carrying out "practices" or preparing "interventions".

Throughout this lecture, attention has been drawn on a number of questions of ethical nature, concerning risk evaluation in particular. Clearly, the answer people give to

these questions affect the way in which they view protection standards for the unborn child.

However, a more relevant question is whether , in this particular field, the unborn child should or should not be protected in *the same* way and with the same dose limits as an ordinary member of the public.

This issue is close to that of the status of the embryo.

Concerning this, B. FELTZ has proposed an interesting approach based on philosophical anthropology. Schematically, there are currently three different concepts with regard to the embryo's status: the "realistic" or genetic one, the "phenomenological" or developmental one and the "idealistic" or relational one.

The "realistic" or genetic approach rests on the continuity of the embryological process since the first day of pregnancy, without any detectable ontological "jump".This approach implies that the embryo has to be considered as a person from the moment of conception.

The "phenomenological" or developmental approach considers the embryo as a "potential" person, becoming more and more a "subject" as the embryological process develops. This often leads the adepts of this concept to adopting a cautious attitude to avoid abuses.

According to the "idealistic" or relational approach, the embryo only becomes a person when there is a parental plan involved.

These three different concepts are irreconciliable in the field of the usual bioethical issues:e.g., research with embryo's, abortion, ..

But the astonishing point is that they all imply a high level of protection for the unborn child when used in the regulatory field. Indeed, even with the most "liberal" approach, the relational one, one cannot deny the exposed woman workers the right and the practical possibility to make parental plans or to give an existential or sacred meaning to the "creation" of a new human being. The embryo's rights here are parallel with the woman's rights.

The cautious requirements of the new BSS directive are therefore totally justified , on ethical grounds.

Accidental exposures

As we have already seen , risks vary from one stage of pregnancy to the next and, even if the dose can be accurately assessed, it will rarely be possible to make a truly reliable estimate as to the existence of the risk, its exact nature or its scale.

The most important thing in this kind of situation is to inform parents as best one can. Providing adequate information implies that the people concerned are informed of the uncertainties and of what is currently seen as the "best estimate" of the risk.

To enable parents to make a correct assessment, it is also necessary to provide points of comparison regarding the spontaneous frequency of the various pathologies involved.

Such an approach would be dishonest if it were used to justify exposures that could be avoided or limited, in connection with practices, but is in my view ethically appropriate as an aid to making decisions when dealing with the consequences of an accident.

Medical exposures

The issues associated with medical exposure have been discussed at European level by the Article 31 expert group, which resulted in a document entitled "Guidance for protection of unborn children and infants irradiated due to parental medical exposures". Although this report is very helpful, it has been difficult to obtain a consensus on several (ethical) issues.

The main problem with this document was that the basic scientific information it contained was sometimes incomplete or too definite. Some experts called for full information (not concealing the existence of uncertainties, such as the risk threshold values, but putting them into perspective). Others felt it was more important to find pragmatic solutions and provide reassurance for the physicians involved. The fundamental question, therefore, was whether it was right for a high-profile document of this kind to leave out, or to present in a veiled manner, certain observations or analyses which might encourage greater caution, and what the ethical and legal implications of such an approach would be. This led, for example, to a discussion on the question of maintaining the 10-day rule (possibly only in certain circumstances), because it is the very beginning of pregnancy which is the most uncertain, not only in terms of ascertaining that there is a pregnancy or establishing the date of conception but also in terms of the nature of any risk or the threshold at which it occurs.

Strategies for the elaboration of an ethical judgment

According to LADRIERE, there are three ways to elaborate an ethical judgment: the decision by a recognized authority, the decision by consensus and the process of what he calls the "reinterpretation". This means that interdisciplinary fundamental reflection work needs to be done to try to find the existential meaning of new situations resulting from technological developments.
Such research work is currently not frequent and the decision by consensus is the most used approach. But the integration of ethical aspects in the elaboration of this consensus is often fragmentary and does not always imply the right persons.

The problem is that a part of society wants to grant a mythical status to Science, by starting with the expert representing it, and in particular if he has a prestigious "halo". "Since our societies want to be democratic, since they no longer (officially) recognize an authority greater than the will of the populations, the only authoritative argument , with regard to what is possible and what is not , will always come, in one way or another, from Science" (I. STENGERS).

If, despite everything, contradictory messages subsist, one then appeals to the *consensus* of the scientists- and often only them- as a condition for objectivity. Doing so , one forgets that the scientific experts, coming from the same melting pot, often share the *same* interpretative language, the same paradigm (a whole of reference presuppositions which are often unconscious). Sometimes consensus should be called "pseudoconsensus".

By way of conclusion, I would say that the existence of ethical aspects is more and more recognized but that the elaboration of a good strategy to formulate ethical judgments still remains a challenge.