GENETIC AND SOMATIC EFFECTS OF IONIZING RADIATION

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NOTE

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ANNEX C

Biological effects of pre-natal irradiation

CONTENTS

Introduction

1. A study entitled "Developmental effects of irradiation in utero" was presented in annex J of the 1977 UNSCEAR report [U2]. It reviewed most of what was known at the time concerning such effects, both in experimental animals and in man, but since the amount of information on the former exceeded by far that on the latter, the study dwelt largely on the experimental animal data.

2. Since then, new information has become available, relating particularly to man, which has highlighted the need to focus discussion on the human experience and to draw on any data that might set more precisely in perspective the effects and risks of irradiation in utero. Moreover, effects such as tumour induction, which have been discussed elsewhere, need to be re-examined in light of the new data if overall risk estimates for man are to be attempted. These considerations have prompted UNSCEAR to undertake the present study entitled "Biological effects of pre-natal irradiation".

3. As reflected by its title, the study is meant to be more comprehensive than the earlier one, including effects such as tumour induction and other long-term sequelae of irradiation which were specifically excluded from the 1977 study [U2]. It is also aimed at drawing conclusions concerning the potential for practical applications, insofar as the data will allow. As the previous study adequately covered the fundamental aspects of the subject, the present one will simply point out how the new data would alter previously drawn conclusions. (Data pertaining to genetic damage induced by irradiation in utero, damage induced before fertilization, and some special effects such as the induction of XO females, are to be found in annex A to the present report.)

4. In the 1977 report [U2] the subject was divided, mainly as a matter of convenience, into general chapters covering the methodological aspects and the mechanisms of action of radiation effects in utero. The substantive data were mostly reviewed in separate sections covering the pre-implantation period, the period of major organogenesis, and the fetal period. Within each section, lethal, teratological, developmental and other effects were considered, information on animals and man being grouped for the purpose of showing qualitative similarities. Such a treatment may not always adequately reflect the complications of the biological models and the variability of the data, but any alternative sub-division of the subject matter would also have its limitations. The format used did, however, offer the advantages of setting the observations in some reasonable sequence with respect to embryonic and fetal development, thereby allowing individual discussions of each major class of effect, by drawing together all observations pertaining to the same stage of development.

5. In the present study, the matter is reviewed again by pre-natal stages in animals and man, in order to facilitate projections across species. This way of ordering the subject does not imply that all such projections may be legitimate and justified. On the contrary, as emphasized repeatedly in the 1977 study, such projections (and particularly the more quantitative ones) should not be undertaken without due regard for the specific characteristics of the biological systems under comparison.

6. As in the 1977 study, the present one will consider separately subjects such as modifying factors, internal irradiation and tumour induction because the relevant information does not apply specifically to any developmental stage but often refers to the whole pre-natal period.

7. Although the main aim of the study is to review the published data with a view to assessing radiation effects in man, this is made difficult by the nature of most of the work available, which is usually directed towards studying the mechanisms of development rather than the radiobiologically important variables, such as dose and time. It should be noted that quantitative information on dose-effect relationships and on the influence of dose rate and radiation quality (which is the most valuable for the Committee's own purposes) is still scanty, particularly at the low doses. Moreover, it is often buried under observations on the pathogenesis of malformations or the morphology of malformed tissues. This, together with the lack of systematic information on species, tissues and effects, may in some instances cause this new study to fall short of its aim. However, in view of the interest that the field continues to command, of the new data available, and of the relatively high risk that exposure of the conceptus in utero might entail under certain conditions, the re-consideration of old data and the discussion of new data is amply justified.

I. BASIC INFORMATION ON THE HUMAN EMBRYO AND FETUS

8. In order to set the following text in perspective with respect to its main aim, it is important to review some basic information on the normal development of the human embryo and fetus. (As a general reference on this subject see [H27].) After a short introduction on the main phases of pre-natal development in various mammalian species, therefore, the present chapter will focus particularly on the human central nervous system (CNS), which is known in man to be most susceptible to radiation-induced pre-natal damage. New data on the gross development and the biochemical events taking place in human ontogenesis will be outlined, together with modern acquisitions on the histogenesis of the brain structures. For the same purpose, a short section will also be devoted to the etiology and epidemiology of malformations in man, as a background to the risk evaluations discussed later (see chapter VIII).

A. THE MAIN PHASES OF PRE-NATAL DEVELOPMENT

9. The development of the conceptus in mammals is usually (and very schematically) divided into three major phases: the pre-implantation phase, the period of major organogenesis and the phase of fetal development. Implantation of the early embryo into the uterine mucosa marks the separation between the first two periods. Although implantation is given in the reports at a certain day post conception (p.c.), it should be remembered that the complete process, from the initial contact of the blastocyst with the uterine wall to its firm attachment through the erosion of the uterine epithelium by the trophoblast and the establishment of a placental blood flow, may last from one to one and a half days in the mouse and a few days in man. A clear-cut separation between the embryonic and the fetal periods is even more difficult since the transition is marked by the end of differentiation and the growth of the newly formed organs in an animal which has attained the characteristic morphological features of the species. It is estimated that by the end of the embryonic period, that is by the 8th post-ovulatory week, the human embryo (measuring about 30 mm in crown-rump length and weighing 2-2.7 g) already possesses more than 90% of the more than 4500 structures described in the adult body [O8].

10. Thus, the conventional limits of the three developmental periods, which are given for various animal species in Table 1, should only be regarded as rough approximations. A tabulation of times for the development of morphological characters in some mammals is also to be found in [S84] and [M57].

11. The division into the three phases mentioned above corresponds approximately with significant differences of developmental events in the embryo. It is also suitable for a description of radiation effects, which are very different in nature and degree within the three phases. For example, death in utero is generally characteristic of irradiation in the pre-implantation phase, while neo-natal death and malformations are associated particularly with irradiation during organogenesis. Irradiation during the fetal stage does not normally lead to gross malformations in the teratological sense, but rather to maldevelopment of tissues and defects of growth, particularly in the CNS and gonads. At high doses, it may lead to death. A systematic description of pre-natal effects of radiation supporting these conclusions is to be found in the 1977 UNSCEAR report [U2]. That report emphasized, by means of examples referring to different animal species and various types of effects, that the outcome of a given radiation exposure in respect to both dose and time is highly dependent on the developmental characteristics of each species. This condition makes it difficult to extrapolate the type and degree of radiation-induced effects across species and to formulate generalized and particularly quantitative conclusions. However, as the developmental physiology is similar, some useful comparisons may be made.

B. NORMAL DEVELOPMENT OF THE HUMAN EMBRYO, PARTICULARLY OF THE CNS

12. In order to assess whether or not there are periods of high susceptibility in human development in regard to any toxic agent, it is important to time the effects induced in relation to the menstrual cycle or to fertilization. A precise mapping of developmental events is still, however, a major unresolved issue in human embryology, because experimental studies are not possible and because one must rely on approximate estimates of the date of conception. Gross measurements, histological investigations and biochemical analyses on autopsy material have usually been employed to this end, although, more recently, direct measurements in vivo have become possible. Up-to-date observations on the early development of the human brain and information on the staging system of human embryology are to be found in several references [M49, O7, O8]. The last of these also provides an annotated list of the chief sources of recent information on the development of various structures of the human body.

1. Gross measurements

13. It should be realized that neither the weight nor the length of an embryo are an adequate guide to developmental status and therefore should not be taken as a "stage", which is a term based on the overall morphology of the embryo and not on any single measurement. Kobyletzki and Gellen [K27] investigated the phase of development of 555 human embryos (morphologic development, fresh weight, crown-rump length) obtained from legal terminations of early pregnancies and correlated it with the duration of pregnancy, age and weight of the mother, parity and number of abortions. They found it impossible to correlate the phase of development in any single case to a reasonable degree of precision and, as a consequence, to predict periods of gestation where the product of conception might be at a higher risk of exogenous embryotoxic agents.

14. The normal and abnormal development of early human embryos was also studied by Nishimura and collaborators. In a first publication [N5] containing data on 1213 intact embryos, standards of normal development (with respect to crown-rump length, body weight and external form) were established which were thought to be more reliable than the standards usually cited. Remarkable variation was noted between clinical age and these growth indicators. It was concluded, therefore, that the general developmental stage was more reliably established by such objective parameters than by the clinically established age. Dead embryos were more frequent in women with genital bleeding during pregnancy. The prevalences of externally malformed embryos were 2.35% (44/1870) at Carnegie stages [O8] 16-18, and 2.12% (46/2169) at stages 19-23 [N14]. Malformations observed included exencephaly, cyclopia, myeloschisis, cleft lip and various limb abnormalities. It was noted that the incidence of most of these defects was far higher than that observed in newborn infants. In a second paper [N6] reporting on 90 normal specimens from healthy pregnancies at Carnegie stages 7-13, they correlated clinically assessed age and growth and found remarkable individual variability in embryos at stages 11-13, but an overall accordance with observations published by other investigators.

15. Jakobovits et al. [J9] also addressed the problem of establishing reliable standards of the rate of growth of human embryos and fetuses. The material examined comprised 354 embryos and fetuses ranging from 20 to 200 mm crown-rump length, obtained by therapeutic abortion in three different countries. Comparison of the measurements taken with the widely used standards of Streeter [S55] (see Table 2) indicated that the discrepancy observable at the embryonic stages diminishes gradually into the fetal period and eventually becomes quite insignificant. The discrepancies may be explained on the ground that Streeter's material [S55] contained data on a significant number of spontaneous abortions in the early embryonic period when the fetal age may be misjudged by the possible occurrence of menstruation after fertilization. The embryos would then be judged to be younger than their real age. When the number of embryos below the standard morphological age for their ovulation age is compared with the number of those above standard, it is found that the morphological development of a large proportion of malformed embryos is below standard [N14]. The paper by Jakobovits et al. [J9] discusses the sources of variability between and within series and concludes that the standards of intra-uterine growth must be considered of limited value in correlating age and measurements in single cases.

16. Skidmore [S43] also examined the crown-rump length of 483 fixed human embryos (Carnegie stages 6-23) and calculated the median and predicted mean length. The results were compared with those of others and found to be in good agreement. Weight standards for the whole body and organs in human fetuses below 500 g (or 200 mm of crown-rump length) were described by Tanimura [T15].

17. In a study of a few hundred staged human embryos examined for a variety of gross and microscopic morphological features, Moore et al. [M30] investigated the significance of a relationship between estimated post-ovulatory age and size or maturity of the embryos, as well as the timing of the development of major landmarks. They concluded that the embryo develops to 1 mm at the 28th post-menstrual day, after which its crown-rump length increases at the rate of approximately 0.7 mm per day up to 80 mm. Coital and post-ovulatory ages estimated from menstrual data correlated significantly ($P < 0.001$) with crown-rump length and the Carnegie stage system. However, the post-ovulatory age, even after adjustment for lack of synchrony between menstrual period and time after ovulation, showed high variability (standard deviation, 10.5 days) indicating poor predictability for single specimens. On the other hand, morphological features appeared within a narrow interval. The data showed that during the portion of embryogenesis studied (up to about 100 days of post-ovulatory age), there is a close synchrony of events, each of which occurs very rapidly in an apparently stereotyped sequence with little statistical variation. The time of normal development may be determined accurately if the date of a single or most likely fertile coitus is known. To assess embryonic age in individual cases on the basis of menstrual data, however, is a very unreliable system of dating.

18. With the development of new ultrasonic techniques, it is now possible to measure in vivo embryonic development as a function of pre-natal age in order to compare such findings with length/age tables in current embryological usage and to score for the presence of congenital malformations [C21, C22]. Drumm and O'Rahilly [D16] made one such comparison on a sample of 44 normal women studied between 33 and 86 days from ovulation. They concluded that, in the embryonic period proper (i.e., up to 8 post-ovulatory weeks), the measurements in vivo give a slightly greater length for a given age than those normally assigned in embryology, possibly due to differences between in vivo and post-mortem length or to imprecision in estimating the data from anamnestic findings. At later stages in the period investigated (early fetal) a good correspondence was found between in vivo ultrasonic and morphological measurements on fixed specimens.

19. Dobbing and Sands [D17] analysed, as a function of pre- and post-natal age, the changes of biparietal diameter, head circumference and brain weight in man. The shape of the curves was shown to be different for each parameter and the differences were compared and justified. The significance of these findings in relation to limiting influences, such as nutritional deficiencies, and to the capacity for recovery, was discussed. Jordaan [J7] also investigated the relationships between body and brain weight as a function of intra-uterine age in man and showed that the ratio of brain/body weight is a useful index for appraising the quality of growth in cases at risk of intra-uterine growth retardation.

20. Burdi et al. [B14] examined organ weight patterns in the course of human fetal development. They studied 80 singleton abortuses assessed as being morphologically normal and typical for age, covering a period between 13 and 31 weeks of fertilization. They obtained regression formulae characterizing the growth of 10 selected fetal organs relative to total body weight (Table 3). According to these authors, fetal body weight (rather than the more commonly used crown-rump length or age since last menstruation) appears to be the best reference parameter for assessing the growth of the various organs and for working out quantitative formulae for such growth. Data analysis showed that, of the 10 organs monitored, the brain was the organ the weight of which is most highly correlated with the changes of either crown-rump length or total body weight.

21. In a series of 72 autopsies, Friede [F8] timed some readily identifiable histological landmarks useful for dating cerebellar development between 24 weeks of gestation and 13 months of life and compared histological events in the cortex with overall weight data for the cerebellum. This work shows that by the 9th post-natal month the human cerebellum has attained only 50% of its adult weight, and up to about this time active growth of the molecular cell layer takes place to its adult dimension. The doubling of the cerebellar weight after the 9th post-natal month is probably due more to a continuing process of myelin formation in the white matter than to continued cell proliferation in the cortex.

22. Table 4, taken from O'Rahilly [O8], summarizes information of a quantitative and morphological nature on the sequence of developmental stages in the human embryo. The author, among other points, emphasizes that the most useful single measurement for assessing the age of an embryo or fetus is the crown-rump length, which is found to agree closely with that determined ultrasonically [D16]. Alternatively, the greatest length of the embryo may be used, which is a practicable measurement from 2 post-ovulatory weeks (stage 6) through the remainder of the embryonic and also in the fetal period [O11]. The 23 stages mentioned in Table 4 refer to the embryonic period only, because no staging system has yet been devised for the fetus. Finally, embryonic ages are now expressed in post-ovulatory weeks or days; little or no attention is paid in modern embryological work to menstrual data.

23. The ectodermal tissue that will eventually form the various brain structures may be identified in the human embryo at about 6 days after conception [O7]. It appears as a neural plate which develops into a neural groove at about 18 days. By day 20 [M49] the three major portions of the brain (the prosencephalon, the mesencephalon and the rhombencephalon) can be identified. The neural groove becomes a neural tube at about 22 days p.c. The open ends of this structure soon close. As the tube grows in length and thickness, it flexes, particularly at the rostral end, while other structures (otic vesicle, optic vesicle) begin to appear. By stage 15 (about 5 weeks p.c.), the neural tube is well differentiated and the embryonic cortical zones, which will finally give rise to the cerebral hemispheres, begin to form [S21].

2. Cellular phenomena

24. The cellular events leading to the formation of the brain cortex in mammals have now been described in considerable detail [S21]. In order to understand these events it is necessary to have in mind the morphology of the embryonic cortical zones mentioned in the previous paragraph and shown in Figure I. The following zones may be recognized: (a) a ventricular zone, made by the ventricular cells which replace the spongioblasts and the germinal cells; (b) a marginal zone, the outermost cell-sparse zone from which cell nuclei are excluded during their movements; (c) an intermediate zone, developing between the previous two, as postmitotic neurons move outwards and afferent axons grow between regions: and (d) a subventricular zone, situated at the junction between the ventricular and the intermediate zones, containing cells giving rise to the macroglia and special classes of neurons.

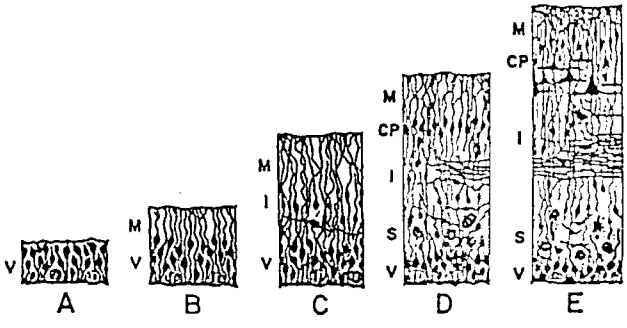

Figure I. Semi-diagrammatic drawing of the development of the basic embryonic zones and the cortical plate. Abbreviations: CP, cortical plate; I, intermediate zone; M, marginal zone; S, subventricular zone; V, ventricular zone. [S21]

25. According to the descriptions of Sidman and Rakic [R23, S21], which integrate previous observations, the whole process of cell migration and cortical development in man may be summarized in a number of stages, as in Figure II (approximate gestational ages in weeks given in parentheses):

Stage I (7-10). This is characterized by the initial formation of the cortical plate, starting with the migration outward of post-mitotic ventricular cells to form a new accumulation of cells (neocortical plate) which is several layers of cells deep by the end of the stage.

Stage II (10-11). There is a primary condensation of the cortical plate which becomes thicker and more compact. Neurons in the plate are immature and their elongated axis is oriented perpendicularly to the surface of the cortex. Migration of cells to the cortical plate from the ventricular zone through the fibre-rich intermediate zone slows down by the 11th week.

Stage III (11-13). The cortical plate becomes subdivided between an external zone of cells with densely packed nuclei and an inner zone with widely spaced large nuclei. The maturation of early migrated cells and the addition of new immature neurons from a new wave of migration accounts for this bilaminate structure of the cortical plate.

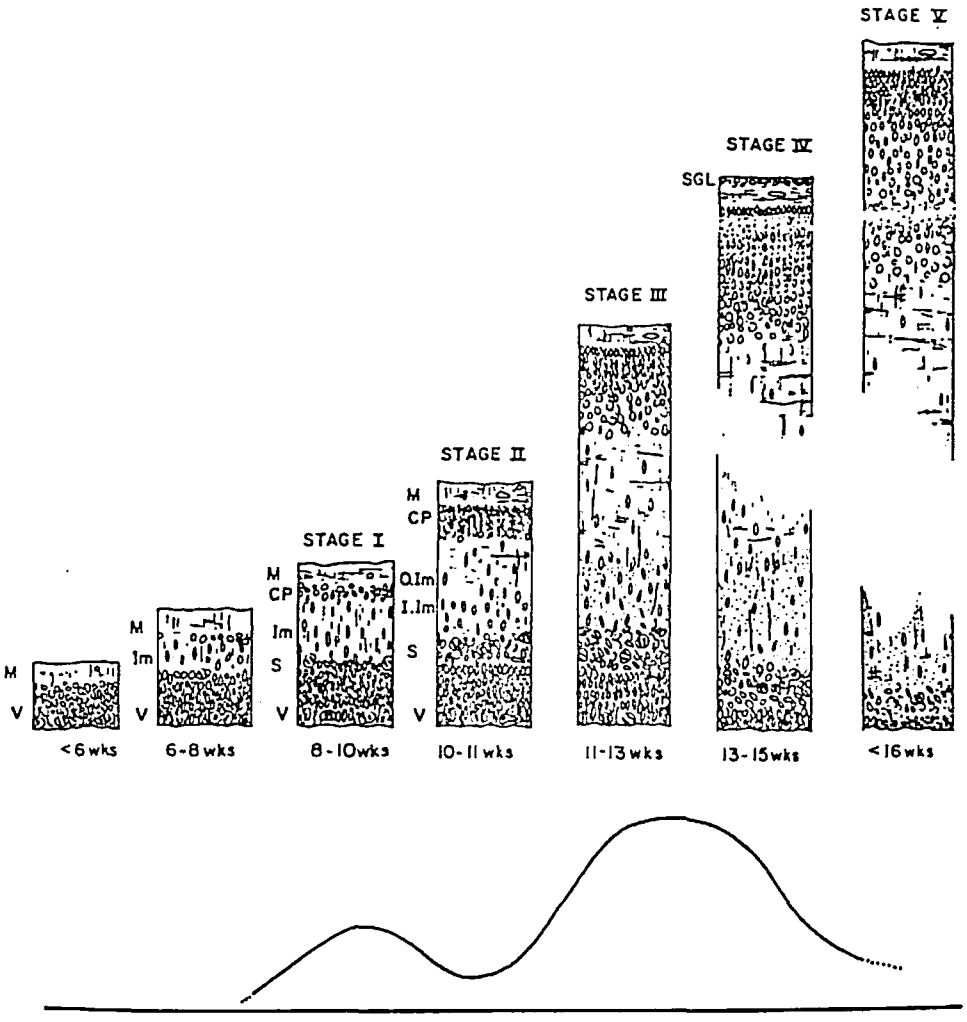

Figure II. Semi-diagrammatic drawing of the human cerebral wall at various gestational ages listed in fetal weeks below each column. The stages refer specifically to an area arbitrarily chosen midway along the lateral surface of the hemisphere. Because there is a gradient of maturation, as many as 3 of 5 stages of cortical development may be observed in different regions of neocortex in the same fetal brain. The extreme right column shows a large interruption in the intermediate zone to signify that the full thickness of the cerebral wall has increased compared to earlier stages. The curve in the lower part of the figure represents roughly the time and relative magnitude of the waves of proliferation and migration of neuronal cells. Abbreviations: CP, cortical plate; Im, intermediate zone; I.Im and O.Im, inner and outer intermediate zones, respectively; M, marginal zone; SGL, subpial granular layer; S, subventricular zone; V, ventricular zone; wks, age in fetal weeks.
[S21]

Stage IV (13-15). There is a progressive thinning of the ventricular zone as many of its cells move outwards and less of the remaining continue to divide. The cortical plate becomes at the same time more homogeneous in appearance, due possibly to an enlargement of the neurons. As relatively few cells enter the cortical plate, its separation from the intermediate zone appears sharper. The intermediate zone has in the meantime become over 5 mm thick.

Stage V (16-after birth). This stage, lasting well into post-natal life is not precisely defined and includes a large range of interrelated developmental events. There is controversy as to the amount and length of neuronal migration during this stage. Although proliferation of the ventricular zone is decreased by the fifth month, many neurons which had been generated earlier are still in the process of migrating to the cortical plate. There is evidence that the subventricular zone persists as a germinal area and continues to produce medium-sized and small neurons as well as an increasing number and variety of glial cells, and that these processes of cell production may continue up to the middle of gestation. Cell migration, on the other hand, may continue throughout the first year of extra-uterine life, but the extent and the timing of these processes of cell production and migration are still uncertain.

26. The curve in the lower part of Figure II is meant to represent, roughly, the two waves of proliferation and migration of neuronal cells, the first one being of shorter duration and involving less cells than the second. These waves are taking place in the human cerebral cortex around stage I and stages II-IV, respectively.

27. In the developing cortex of the cerebellum, cell relationships are very similar, but the migration of granular cell neurons proceeds from the external surface inwards past the dendrites and somas of the Purkinje cells, guided by the radial arrangements of the Bergmann glial fibres. There are also other special migration patterns applying to some zones of the CNS.

28. It is not yet clear whether cell migration might involve the same mechanisms during the initial stages of cortex development, or during later stages when cells move along distances of several millimetres. However, it is now well established that migrating neurons find their way to the cortex by following the radially oriented fibres of the glial cells as guides. The later generated cells take positions external to those of their predecessors and the final position of cell elements along the radial sectors may be influenced by afferent axons. Cell surface properties may be involved in controlling migration, but their nature still remains undefined [R24].

29. Another paper by Rakic [R9] added important details to the basic phenomena described, which are of significance in respect to the specificity of origin and interconnections of neurons. A systematic analysis of the neuron division and migration carried out by ^{3}H-thymidine labelling in the Rhesus monkey has made it possible to establish that successive generations of neurons originating in a given location at the ventricular surface migrate along the same glial fibres and settle themselves eventually in the same radial cortical column. Thus, the glial fibres, in addition to directing neuronal migration, may be of importance in preserving the topographical, and therefore functional, relationship of clonally related neurons. They are also important in reproducing the mosaicism of the ventricular zone in which neurons originate at the level of the expanded surface of the cerebral cortex. It is not known whether the killing of glial cells may impair the orderly migration of the neurons. In summary, although migration of cells from one part of the body to another is a common phenomenon in ontogenesis, there are unique features associated with cell migration taking place in the developing brain. First, as it only occurs after the last cell division, it involves neurons that will not reproduce any longer. Secondly, it involves active cell movement following selected pathways along the glial fibres. Finally, it exhibits precise space and time dependencies [R24].

30. In addition to neuroblast division, migration and differentiation, neuronal death has been shown to play a role in shaping the final morphology and function of the adult brain. Various types of programmed neuronal death have been described in various animal species [C17]. One is related to the target area to be connected by certain types of neurons and is to some extent adjustable to the size of this area; another is related to the elimination of errors that may occur in connecting various areas of the brain; a third relates to the selective response of neurons to circulating hormones; and a fourth is under tight genetic control. Although there is doubt whether, and to what extent, all these types may be present in the developing mammalian brain, it appears that programmed death of neurons is directed to the adjustment of each neuronal population to the size or functional needs of its projection field and to the elimination of neurons whose axons have grown to the wrong target or to the wrong region within the target area. Other regressive events leading to the elimination of some neuronal connections initially formed, without death of the parent cell, are also known, and they appear to be directed to the fine tuning of neuronal wiring. All these phenomena are now recognized to play a major role in the final maturation of the CNS, but nothing is known yet about their possible relationship or importance to radiation-induced cell killing.

3. Biochemical development

31. Brain maturation may also be followed by mapping of biochemical events as a function of time. Howard et al. [H14] studied the content of desoxyribonucleic acid (DNA) in 28 human fetal brains at between 10 and 31 weeks of gestation from surgical specimens obtained in Sweden and in the United States. The cerebrum and cerebellum were analysed separately and, in addition to DNA and ribonucleic acid (RNA), cholesterol was also estimated as an index of the biochemical maturation of the organs. The total content of DNA in the cerebrum increased exponentially between 10 and 14 weeks of gestation, after which the rate gradually declined to a linear function; there was no sign that growth would stop up to 31 weeks of gestation (Figure III). By contrast, the DNA content of the cerebellum kept increasing exponentially throughout the same period (Figure IV).

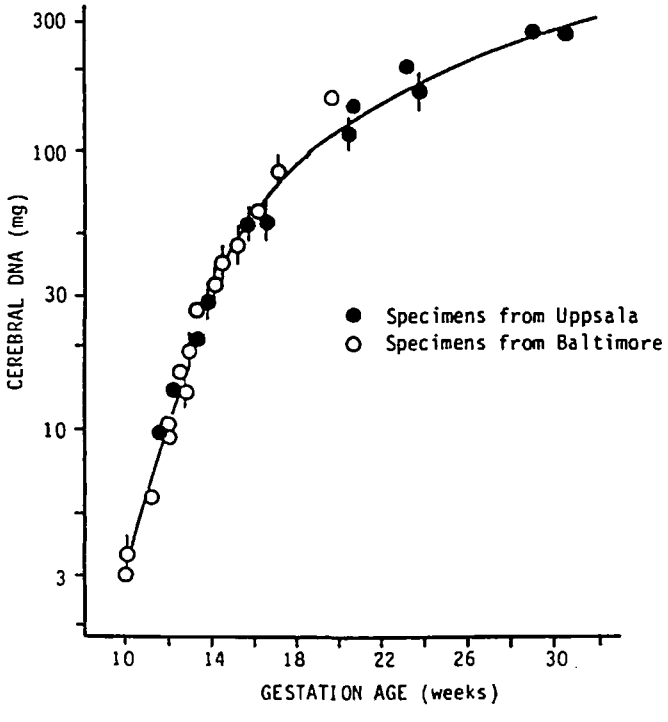

Figure III. The increase of total cerebral DNA during development of the human fetus. Ordinates: DNA in mg on a logarithmic scale; abscissae: age in weeks from the onset of the last menstrual period, estimated from body weights according to Streeter. Each circle represents one brain. Data from [H14].

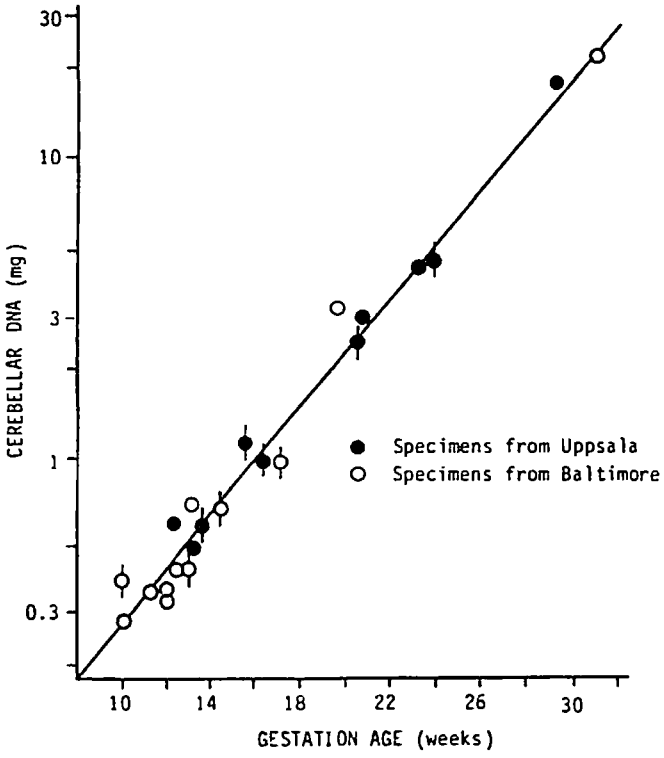

Figure IV. The increase in total cerebellar DNA during development of the human fetus. Each circle represents one brain. Data from [H14].

On the assumption that the increase in DNA may roughly correspond to an increase in the number of nuclei, the different patterns of growth of the cerebrum and cerebellum were interpreted to show that in some areas of the cerebrum cell division may be terminated earlier than in the cerebellum. These data also showed, in agreement with morphological studies, that in man there may be considerable post-natal cell division of neuronal precursors.

32. In the cerebrum, total RNA increased with DNA and tissue weight. The RNA/DNA ratio, taken to be proportional to the mean RNA per cell, showed no appreciable change at the time of maximum DNA growth between 10 and 14 weeks of gestation. Thereafter, as the rate of DNA accumulation declined, the RNA/DNA ratio rose progressively, probably reflecting an increased cell differentiation. The ratio of tissue weight to total DNA, considered to be proportional to mean cell size or mean cell territory, increased exponentially up to 30 weeks. The cholesterol/DNA ratio, i.e., the amount of cholesterol per cell, increased exponentially throughout the age range studied, in good relation with the increase in cell membrane surface area associated with the developing dendritic arborization. Consistently different patterns for all the above quantities were observed in respect of cerebellar development.

33. The quantitative growth of the human brain was also followed by Dobbing and Sands [D12]; 139 human samples, ranging in age from 10 weeks gestation to 7 years post-natal, were studied, together with 9 adult brains. The whole brain and the three major regions (forebrain, stem, cerebellum) were examined separately for weight, DNA, cholesterol and water content, in order to describe quantitatively the spurt in brain growth. The weight of the whole brain and its parts followed a sigmoid trend with time, with a cut-off point between 18 and 24 months of post-natal life for whole brain and forebrain. The cut-off point for the cerebellum was a little earlier than that of the whole brain. The cerebellum appeared to start growing later and to come to a plateau earlier than the rest of the brain. Cellularity (as expressed by the amount of DNA per g of tissue) in the forebrain and in the stem decreased as a function of age up to birth, due to disproportionately rapid growth of cell size, cell branching and myelination. In the cerebellum, however, cellularity increased until after birth, owing mainly to a rapid rate of cell multiplication.

34. Total cell number increased in an approximately sigmoid fashion as a function of time in the whole brain and all its regions. In the whole brain the cut-off point marking the levelling-off of the sigmoid curve occurred at around 18 post-natal months. Cerebellar cell growth started later and finished earlier, while the forebrain curve bent over at about 2 years of age. Figure V shows a semi-logarithmic plot of total DNA (proportional to total cell number) in the human forebrain between about 10 weeks of gestation and 4 months post-natal. The first of the two growth phases was thought to reflect rapid neuronal proliferation, the later, shallower, part was attributed to glial proliferation. The sharp bend of the curve at about 18 weeks of gestation does not imply that neuronal proliferation is stopped completely at this time, nor that glial cell division does not occur before, but rather that the rate at which the two processes proceed is sufficiently different to be reflected in the overall curve. The similarity of these data with the earlier ones reported by Howard et al. [H14] and shown in Figure III is remarkable. Figure VI summarizes, for the three different brain regions, the values of total DNA content as a function of fetal and post-natal ages, expressed as percentage of adult DNA values.

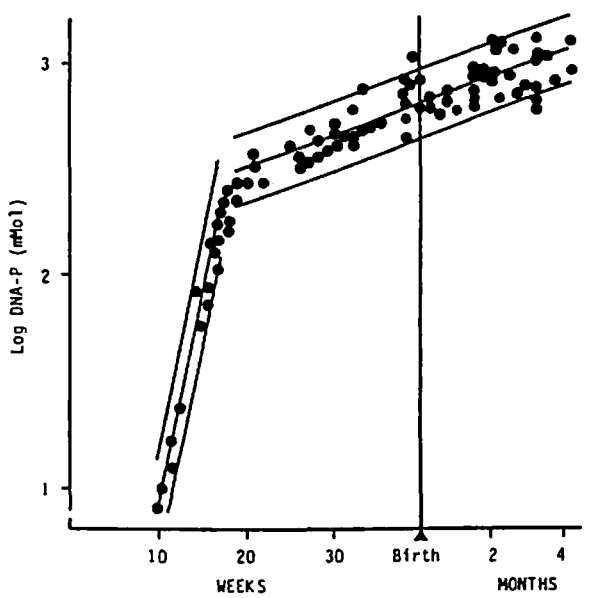

Figure V. Total DNA-P (proportional to total number of cells) in the human forebrain from ten gestational weeks to four post-natal months, showing the two-phase characteristics of pre-natal cell multiplication. [D12]

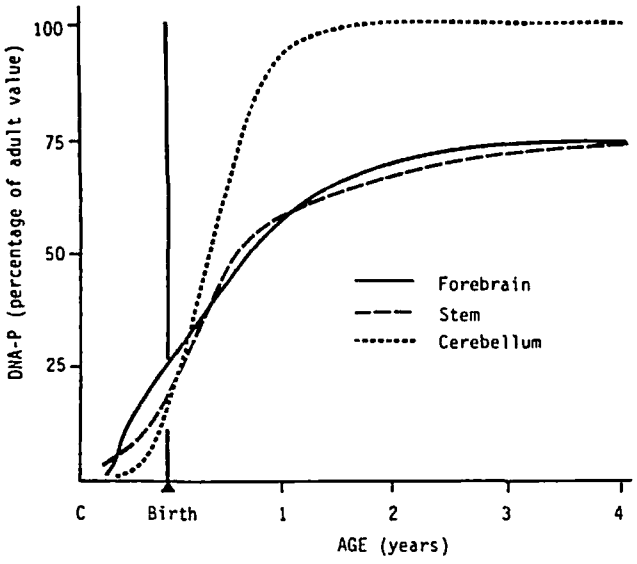

Figure VI. Relative values for DNA-P (proportional to total number of cells) in three human brain regions. [D12]

35. The cholesterol content is taken as an index of myelination. For cholesterol, the growth spurt was less sharply defined and extended for whole brain, forebrain and stem well into 3 or 4 years after birth, possibly up to 5 years. The cerebellum seemed to accumulate this substance faster compared with other regions of the brain.

36. The paper by Dobbing and Sands [D12] adds considerably to the previous one by Howard et al. [H14]. It establishes a growth spurt for human brain development centred around the time of birth, but extending from mid-pregnancy well into the second post-natal year and beyond. This is at variance with what happens in other species: for example, the growth spurt is essentially post-natal in the rat, pre-natal in the guinea pig and perinatal in the pig. Data referring to the timing of the brain growth spurt in various mammalian species taken from another paper of Dobbing [D13] are shown in Figure VII. Dobbing and Sands [D12] commented on the length of human fetal brain development in comparison with that of other species and on the timing of the various processes in relation to noxious influences such as malnutrition. They specifically drew attention to the striking correlation between the high rate of multiplication of neurons between 10 and 18 weeks of gestation and the period of maximum sensitivity for radiation-induced small head size and mental retardation shown by Miller and Blot [M20] in children exposed in utero during the bombing of Hiroshima and Nagasaki. Mole [M10] also emphasized this correlation. Although it has long been known that the time of major organogenesis of a structure coincides with its maximum sensitivity to irradiation [U2], the observation of Dobbing and Sands [D12] is important in human radiobiology because it provides a cellular basis for the occurrence of mental retardation.

37. Dobbing and Sands [D18] investigated the mechanisms for the vulnerability of developing rat brain. Starting from the notions that the brain is especially vulnerable to growth retardation during the growth spurt, and that any deficit imposed at this time cannot be recovered at later stages, even if the

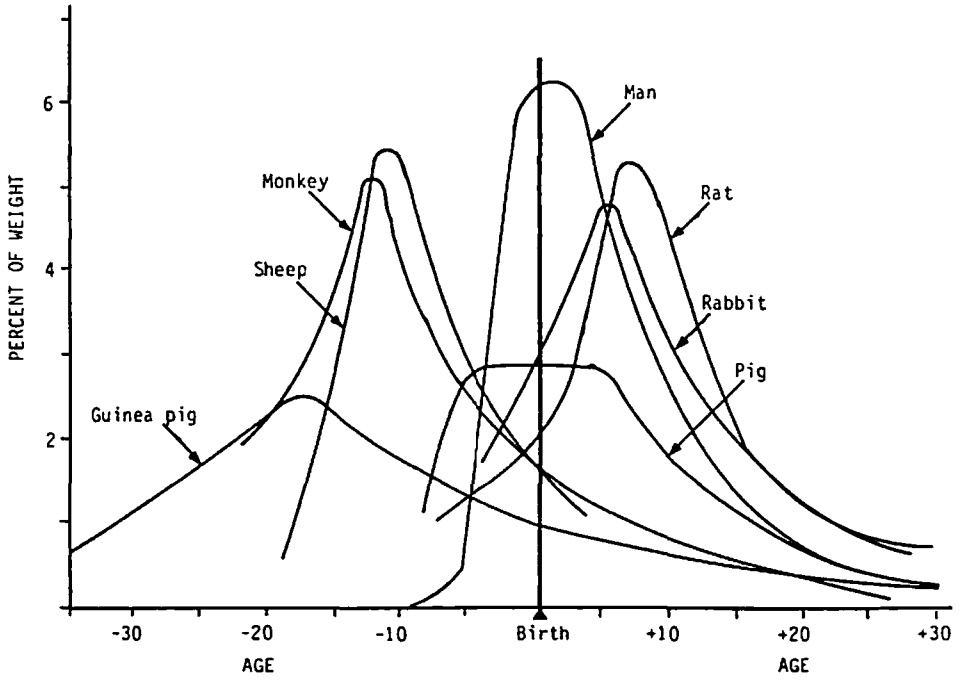

Figure VII. The total brain growth spurts of 7 mammalian species expressed as first-order derivative curves of the increase in weight with age. It should be noted (Figure VI) that different regions of the brain develop at different times. Weights are expressed as weight gain as a percentage of adult weight for each unit of time. The units of time for each species are as follows: guinea pig, days; rhesus monkey, 4 days; sheep, 5 days; pig, weeks; man, months; rabbit, 2 days; rat, days. [D13]

restrictive action is over, they attempted to study whether growth retardation might be due to a chronological delay of the growth spurt complex or, instead, to a lesser extent of the growth spurt occurring at a fixed chronological time. They examined, as a function of time before and after birth, the changes in weight, DNA and cholesterol concentration in the brains of either normal or undernourished growth-retarded animals and showed that these components of the brain growth spurt were determined by the chronological and not by the developmental age. Thus, the brain has a once-only opportunity to grow correctly and when such opportunity is restricted the damage directly or indirectly induced may not be recovered at later stages.

38. In a review of the effects of early nutrition on CNS growth, Winick et al. [W13] pointed out that the series of critical periods occurring during brain development make this organ particularly sensitive to lack of nutrients. During the post-natal proliferative growth of some mammalian species, including man, malnutrition may interfere with cell division. In the pre-natal life of the rat, maternal under-nutrition blocks cell division in the fetal brain, whereas uterine vascular insufficiency does not. This suggests that there may be different types of intra-uterine growth failure. In man, severe malnutrition retards placental cell division and may affect fetal brain development. However, the study of these effects is difficult because of the lack of appropriate biochemical markers to assess fetal growth in utero. A later chapter by Dobbing [D13] also reviewed the problem of brain vulnerability to the lack of nutrients in relation to structural development.

39. In a short communication, Edwards [E7] pointed out that true microcephaly is difficult to produce experimentally because most noxious agents retard general body growth as well as brain and skull growth. However, some noxious agents such as certain viruses (rubella, cytomegalovirus), antimitotic agents, hyperthermia and radiation, act by destroying proliferating neuroblasts, and the surviving cells are unable to make up for the deficit through additional divisions. The subsequent proliferation of glial cells and production of myelin are also reduced in proportion to the neurons surviving. The net result is a brain which is normal in shape and histology, but deficient in size and possibly in function. The lack of correlation between the size of the brain and the deficit in mental activity has also been commented upon by Dobbing and Sands [D18].

40. Erzurumlu and Killackey [E5] discussed the presence of critical and sensitive periods in neurobiology, extending the concept from the morphological to the functional development of the CNS. In their view, the word "critical" should be reserved for normal development, to denote those periods during which the action of a specific external or internal condition or stimulus is required for the normal progress of development; "sensitive" periods, however, are times in development during which a given system is highly susceptible to the effects of harmful agents. This term refers, therefore, to the realm of teratology. In connection with such critical and sensitive periods, the authors discussed the effects of nutritional and hormonal deficiencies, of the exposure to toxins or other harmful agents, and of stimuli deprivation during development. They showed that it is at the time of the fastest rate of growth of a given structure that vulnerability is at its maximum. They also illustrated, with practical examples, a general theoretical approach to the problem of critical periods and an analysis of a system's organization and rate of development in relation to such periods.

C. EPIDEMIOLOGY AND AETIOLOGY OF HUMAN ABNORMALITIES

41. To keep the findings in a proper perspective when discussing radiation-induced developmental defects in man, it is important to know the natural birth prevalence of congenital anomalies. The brief outline of the epidemiology and aetiology of congenital anomalies given below is based on general references [C11, C14, C18, I5, K32, L4, M45, N9, N10, S38, U5, V5, V8] which contain compilations of numerous original literature reports, in addition to original material. No effort is made to review individual reports independently, since the object of the exercise is simply to provide some indications of the nature and order of magnitude of the baseline occurrence of human anomalies.

1. Terms and concepts

42. The term "congenital anomaly" is used by the World Health Organization (WHO) [W26] in the International Classification of Diseases (ICD) to designate structural (or morphological), biochemical and functional developmental disturbances which occur in the fetus (in this context a conceptus, irrespective of the duration of pregnancy prior to the complete expulsion or extraction from its mother, is called fetus [W26]) from conception until birth, and which are present at birth, whether detected at that time or not. The term "congenital anomaly" is a very broad one and includes a number of categories of developmental disturbances, as follows: congenital abnormalities; genic defects, including inborn errors of metabolism and chromosomal aberrations; late intra-uterine infections and consequent damages, the so-called fetopathies; idiopathic intra-uterine growth retardation; immunological diseases, e.g., mother-fetus rhesus blood group incompatibility; mental retardation and behavioural deviations; congenital defects of the sense organs and other handicaps; congenital hernias; and tumours.

43. The congenital abnormality is a structural defect, gross or microscopic, that is present at birth, whether diagnosed at that time or not. Recently, a pathogenetically oriented and practical classification has been recommended [S77] for defects of morphogenesis (i.e., congenital abnormalities) as follows:

(a) Malformation: a morphological defect of an organ, part of an organ, or larger region of the body resulting from an intrinsically abnormal developmental process;

(b) Disruption: a morphological defect of an organ, part of an organ, or a larger region of the body resulting from the extrinsic breakdown of, or an interference with, an originally normal developmental process;

(c) Deformation: an abnormal form, shape, or position of a part of the body, caused by mechanical forces;

(d) Dysplasia: an abnormal organization of cells into tissue(s) and its morphological result(s), that is, the process (and the consequence) of dys-histogenesis.

44. Pathogenetically, it is necessary to separate isolated and multiple patterns of morphological defects within congenital abnormalities [C18]. For example: isolated cleft lip, with or without cleft palate, may represent a homogeneous group of congenital abnormalities of similar origin. However, cleft lips with other, different, congenital abnormalities may occur as part of multiple congenital abnormalities in a number of diseases of different origin. Thus, the combination and joint evaluation of isolated and multiple congenital abnormalities is the cause of serious confusion both in research and in clinical practice.

45. Isolated congenital abnormalities are the consequences of developmental disturbances of single localized errors in morphogenesis. This category involves single, complex (more abnormalities in one organ or organ system), sequential (one defect with its subsequently derived secondary defects in later morphogenesis) and polytopic field defects (a pattern of defects derived from the disturbance of a developmental field). A multiple congenital abnormality is a concurrence of two or more morphogenetic errors of different sites in the same person. Within this category, syndromes (patterns of multiple anomalies thought to be causally related), associations (non-random patterns of two or more anomalies presumed to be pathogenetically related) and random combinations are usually distinguished.

2. Birth prevalence of congenital abnormalities

46. The baseline birth prevalence of all congenital abnormalities in man is difficult to assess for a variety of reasons. First, as is well known, the affected newborns seen at birth are only the survivors of pre-natal selection, i.e., of a much greater number of abnormal fetuses which occurred at one or another stage of prenatal development. Therefore, only their point prevalence at birth (and not time incidence) can be determined for a majority of congenital abnormalities: recently, the former has been designated as birth prevalence. Second, some classes of abnormalities (e.g., coarctation of aorta, teeth abnormalities) cannot be scored at birth or are fewer at birth than after a few years, i.e., their point prevalences would be higher by 50 to 100% if cases could be followed up for a few years. However, the rate of these late-diagnosed cases may permit estimates of prevalence at birth. Third, the prevalence of other abnormality types (e.g., ventricular septal defect or undescended testicle) may be higher at birth than later, owing to spontaneous recovery. Fourth, a large group of congenital abnormalities such as congenital dislocation of the hip or phenylketonuria has only a "theoretical" birth prevalence because, due to neo-natal screening, at least in some countries, the development of these conditions may be prevented by special treatment or diet. The method of scoring (birth or death certificates, hospital records, special anomaly registries, surveillance or monitoring systems), the variable sizes of the samples, the variability of the definition and classification of congenital anomalies and the diagnostic criteria between different observers, differences in reference unit (affected individual or congenital anomaly) are important factors influencing the estimates. To these should be added other confounding demographic factors (age, parity), ethnic group, the family genetic background, and the socio-economic characteristics of the parents (e.g., social class). Finally, there is the additional variability related to the geographical location, the season of the year, and the time span covered by the investigators. All these factors explain the difficulty in comparing the figures reported in the literature.

47. It is not surprising, therefore, that the figures found or cited in the references given [C11, C14, C18, I5, K32, L4, M45, N9, N10, S38, U5, V5, V8] are very variable from a low of 1.0% of all liveborns to a high of 8.5% in total births (i.e., still- and live-births) in the United States [M45]. Recent Hungarian estimates suggest that, for the group of conditions designated by ICD codes 740 to 759 (in chapter XIV of the ICD entitled "Congenital anomalies"), the prevalences are 6.1% and 6.0% in total births and live-births, respectively. If the entities covered by the ICD codes 550 (inguinal hernia), 553 (umbilical hernia) and 227-228 (congenital tumours)—which many teratologists consider as congenital anomalies—are included, then the figures become 7.2% and 7.3%, in live- and total births, respectively. In the prospective United States study [M45] which aimed at as complete an ascertainment of all congenital anomalies as possible (and in which the liveborn children were followed up to one year of age), the prevalence (including ICD entries 740-759, 550, 552, 227-228) was 8.5% of total births.

48. It is worth stressing the fact that in both the Hungarian and the United States data, musculoskeletal and skeletal anomalies are the predominant contributors to the total. Foremost among them, especially in Hungary, is congenital dislocation of the hip. Anomalies of the integument constitute about 10% of the total in the United States and about 1% in Hungary. (For detailed comparisons between the Hungarian and the United States data, see chapter I of the annex A to the present report.) Since a truly representative figure, applicable in a global context, for the prevalence of all congenital anomalies is difficult to arrive at, and in view of the differences existing between different studies (some, genuine differences between populations and some due to different degrees of ascertainment), a round figure of 6% of total births for the prevalence of congenital anomalies with medical consequences, is adopted in the present study, to provide a general frame of reference for discussions. It is realized that this

estimate might become higher if anomalies ascertained later in post-natal life, minor structural blemishes or conditions of little medical significance, low birth weight, mental retardation and other congenital handicaps (not always accurately ascertained) are also included.

49. As a result of progress in epidemiological expertise, birth prevalences of specific types of congenital abnormalities are less variable. In fact, a number of well-defined and easily diagnosed congenital conditions (e.g., cleft lip, spina bifida, anal atresia) have approximately similar birth prevalences in several countries. Furthermore, birth prevalences of different types of congenital abnormalities show a surprisingly narrow range of random fluctuation within the same country or region [I9]. Congenital anomalies are usually listed according to the anatomical structures affected and they are grouped together, although they may have quite different pathogenetic mechanisms. Therefore, a pathogenetically oriented classification system is very much needed.

50. The approximate birth prevalences of all isolated common major congenital abnormalities are shown in Tables 5, 6 and 7. Table 5 is based on a study of a series of consecutive births in 24 centres organized by WHO in 1961-1964 [S38]. In this study, a similar approach was used in all the participating centres and the outcome of a total of 421,781 pregnancies was investigated. The study indicated geographical variations of birth prevalences of congenital abnormalities depending on ethnic-genetic, territorial-environmental, demographic and social (health service, etc.) circumstances. Some congenital abnormalities were not investigated in the WHO study. In these cases, the data of other important epidemiological studies were taken into consideration. These figures are indicated by asterisks in Table 5 and the references are in [C18]. Table 6 shows the data from Hungary where birth prevalences were obtained by the same method (ad hoc epidemiological studies) and by the same staff in a population of 10.7 million between 1963 and 1974. Over this period, the prevalence of congenital abnormalities was studied continuously. The variability of the estimates as a function of time was surprisingly small. Table 8 (modified from a review of Villumsen [V8]) expresses the proportion of congenital abnormalities in various anatomical structures as a percentage of all abnormalities scored, and shows a range of values which is comparable to that seen in Tables 5 to 7.

3. The aetiology of human congenital abnormalities

51. As mentioned earlier, there are a number of ways of classifying congenital abnormalities, e.g., according to their severity (lethal, major, mild, minor), occurrence (common, moderately frequent, rare), teratological manifestation (zygopathy, embryopathy, fetopathy) and aetiology. Their classification according to aetiology, which is considered below, distinguishes between those of simple genic origin; those due to the interaction between hereditary, mainly polygenic, liability and environmental triggering factors; those due to chromosomal aberrations; those brought about by the action of environmental, including teratogenic and maternal, factors; and finally, a heterogeneous class of abnormalities of unknown origin [K32].

52. Mutant genes, autosomal or gonosomal, recessive and dominant, may be the cause of congenital abnormalities, mainly congenital malformation syndromes. The occurrence of each single mutant gene in the population at large may be small, but since these mutant genes are numerous (a few thousand have been described in the McKusick catalogue [M47]), their overall effect in the population may be important. Their variability in penetrance, their rarity, and the differences in clinical severity of their manifestations (expression) account for the difficulties in estimating the frequency of the relevant malformations. Figures between 0.5 and 10 per 1000 births have been provided, but the extreme values may reflect overassignment or incomplete ascertainment of cases to this class. Taking intermediate values of 2.25 per 1000 [C11] or 5 per 1000 children born with a serious disorder [W27], it may be calculated that of the order of 3.8 and 8.3 (i.e., as an average round figure 6%) of all congenital abnormalities may be of genic (or monolocal, monogenic or Mendelian) origin in North America and Europe (2.25/60 = 3.8%; 5.0/60 = 8.3%; average between 3.8 and 8.3% = ~6%). Inclusion of still-births in these estimates would make little change as the major part of congenital abnormalities in the still-born is not of genic origin.

53. To the second class of abnormalities belong all those for which twin and familial studies indicate a complex aetiology explained by the multifactorial-threshold model [C18]. These congenital abnormalities depend on a polygenic disposition interacting with a variety of mostly unknown environmental factors. For congenital abnormalities belonging to this class, the findings pointing to genetic disposition in the various major types and the genetic mechanism underlying their appearance have been discussed by Carter [C11]. According to a WHO study [S38], there are 11 most common congenital abnormalities, that is, those exceeding the rate of 1 per 1000 births. Their detailed break-down is given in Table 5. Comparable data for Hungary, where the situation has been followed carefully, are given in Table 6 [C18]. In this country, for example, the total prevalences of congenital anomalies are 73.6 and 71.8 per 1000 total or live-births, respectively [C19]. Table 6 shows that, for 9 out of the 11 conditions, the most likely explanation is the multifactorial aetiology. The overall point prevalence of these 9 in Hungary is 54.0 per 1000 births, that is 73% of all congenital abnormalities in that country, as shown in Table 6 (54.0/73.6 = 73%). (The figure of 54.0 is obtained by adding the prevalences for: neural tube defects (2.9), ventricular septum defects (2.1), cleft lip ± cleft palate (1.0), congenital hypertrophic pyloric stenosis (1.5), undescended testicles (3.6), hypospadias (2.2), congenital dislocation of the hip (28.0), structural talipes equinovarus (1.3) and congenital inguinal hernia (11.4)). It should be borne in mind, however, that the number of cases with treated congenital dislocation of the hip is extremely high in central and eastern Europe. Recently figures of

the order of about 5 per 1000 for congenital dislocation of the hip have been published in western and northern Europe. Taking this into consideration, the proportion of the 9 isolated common congenital abnormalities becomes 42% of all congenital abnormalities, as a minimal figure (54.0—28.0 + 5.0 = 31.0 out of 73.6 is 42%). Furthermore, only structural talipes equinovarus was evaluated within groups of varus, valgus and other deformities of feet with a figure of 28.8 per 1000 births. Also, about 3.5% of male new-borns have undescended testicle(s) at birth, but nearly 80% of these descend spontaneously within the third month. Finally, some moderately frequent isolated congenital abnormalities (e.g., cardiovascular malformations with a birth prevalence of 5 per 1000 (i.e., one-half of 10.6 minus 2.1)) may belong to this class. Taking all these considerations into account, a total prevalence of 30 per 1000 births, i.e., about 50% of all congenital anomalies, appear to be a reasonable estimate for this class (i.e., 50% of 60/1000).

54. In the third class belong those due to chromosomal abnormalities. The most recent UNSCEAR review of the total frequency of chromosomal abnormalities in newborns (which includes a total of 67,014 karyotyped cases) [U5] points to a birth prevalence of 0.63%. Not all of these would carry visible malformations, but assuming that roughly 50% do, it may be estimated [W27] that the contribution of this class to the total abnormalities in man may be of the order of 5% ($3.1/10^3 \div 60/10^3 = 5\%$). This figure would negligibly increase when still-births are included. It hardly needs to be stressed that chromosomal abnormalities are more frequent early in pregnancy. The review [U5] indicates that about 15% of recognized conceptions will lead to spontaneous abortion (early and intermediate fetal deaths) and about one-third of the aborted fetuses will carry some kind of chromosomal aberrations [H18, H21]. Germinal chromosomal mutations occur in about 5% of recognized conceptions [H22, W26]. The rate of embryonic loss between conception and recognized pregnancy is unknown, but is generally agreed to be very high [K39]: of the order of 40% between conception and 20 weeks of pregnancy [M7] and of the order of 10% between 10 and 18 weeks [G16]. These figures indicate that most conceptual losses must occur before pregnancy has been diagnosed, and often before the first missed period. The occurrence of abnormalities in the spontaneously aborted fetuses is also much higher than that seen in new-borns, which points to the role of fetal death in selecting morphologically and chromosomally abnormal fetuses.

55. The fourth class of abnormalities is that induced by environmental factors, many of which have been suspected (but less often proved) to induce teratogenic effects in utero. The list that follows is taken from Kalter and Warkany [K32], who gave a detailed discussion with references. Among environmentally induced congenital abnormalities, it is worth separating maternal conditions from teratogens, because the importance of the former is probably underestimated, while that of the latter may be exaggerated. Maternal diseases (e.g., diabetes mellitus, phenylketonuria, some hormonally functioning tumours and perhaps hypogonadism) belong to the group of maternal conditions. Maternal infection (e.g., rubella and varicella which can cause embryopathy and fetopathy, and cytomegalovirus and toxoplasmosis, which may damage the fetus) is a condition which is intermediate between the maternal and the teratogenic factors, because the infectious agents may sometimes cause congenital abnormalities without affecting the mother. Considering all maternal factors together, probably 3.5% of congenital abnormalities could be attributed to this group of causes.

56. Among chemicals, pharmaceutical drugs seem to have the greatest practical significance as teratogens. At present, the list of teratogenic drugs involves thalidomide, androgens, anticancer and immunosuppressive cytotoxic and antifolic-acid derivates, synthetic female sex hormones, anticonvulsants (including hydantoin, trimethadion, valproic acid and other derivatives), anticoagulants, large doses of vitamin A and its derivatives, lithium and penicillamine. The mild effect of tetracyclin (teeth discoloration), and the reversible goitre after excess maternal ingestion of iodide or propylthiouracil, have also been discussed. However, the suspicion of teratogenicity for many of these drugs has not been confirmed. Among nutritional factors, the ingestion of alcoholic drinks during pregnancy has the greatest importance owing to the fetal alcohol syndrome. Of a long list of environmental substances, at present only mercury is considered to be a demonstrated human teratogen [K32].

57. Specificity is an important attribute of teratogenesis because nearly all known teratogens cause characteristic multiple congenital abnormalities, i.e., syndromes. Many other conditions or agents may, of course, have non-teratogenic effects on the fetus; for example, hyperthyroidism and lupus erythematoides as well as occupational exposure to anaesthetics may increase the rate of fetal death, while active and passive smoking during pregnancy may cause a significant reduction of weight in newborns. To give an approximate figure for the birth prevalence of environmentally induced anomalies is very difficult because any agent administered in high amounts could be toxic and could retard the development of human fetuses or cause fetal loss. On the other hand, several environmental factors could have a triggering effect on a number of common or moderately frequent congenital abnormalities of polygenic origin. Estimates of the causal role of teratogens vary between 1 and 5% of all congenital abnormalities. Thus, it is estimated that roughly 6% of congenital abnormalities might be caused by environmental (including maternal) factors.

58. In summary, it is clear that, on the basis of their aetiology, the congenital abnormalities can be subdivided into those due to (a) major genes, 6% of the total prevalence of 60/1000; (b) multifactorial causation, 50% of the total; (c) chromosomal anomalies, 5% of the total; and, (d) environmental, including maternal factors, 6% of the total. It can be inferred that about 30% of the abnormalities scored at birth have no known cause at present.

D. CONCLUSIONS

59. The development of the mammalian conceptus may be divided approximately into three major phases: the pre-implantation phase, lasting from fertilization to the settling of the embryo into the uterine mucosa; the phase of major organogenesis, which extends in man to approximately the 8th week post-ovulatory; and the phase of fetal development, lasting from about 9 weeks until birth. The timing and developmental details of each phase, however, differ greatly in various species and may considerably influence the outcome of a given radiation exposure. Quantitative extrapolations across species are therefore unwarranted.

60. The developing human brain appears to be vulnerable to irradiation. In order to assess the existence and extent of special sensitivity periods, it is important to map the development of the nervous system in relation to time. To this end, time after ovulation is a more reliable parameter than time after menstruation. There is, however, no single quantitative parameter to describe the developmental age of an embryo or fetus. Although the length of the embryo is statistically well correlated with its age and developmental status, it is, in any single case, of limited value for predictive purposes. It has, however, the advantage of being directly measurable in vivo using non-destructive ultrasonic techniques. These give information that is in close agreement with measurements taken on embryological samples. There are standardized systems of staging embryonic development, based on the overall morphology of the embryo, by which the major landmarks of organogenesis may be characterized with sufficient precision.

61. The formation of the human brain has recently been described in considerable detail according to the major stages of organogenesis, the cellular kinetics of the developing structures and the overall biochemical maturation of the various structures as a function of time. The major features are: the division of undifferentiated neural cells in the ventricular zone; the migration of post-mitotic cells to the cortical zones according to precise sequences of time and programmed spatial arrangements; the establishment of functional connections between neurons and glial cells and between neurons in various areas of the brain; and the presence of selective mechanisms to adjust the size and functions of the nerve cells in relation to their areas of projection and to neuronal wiring. All these phenomena and their temporal sequence determine, to a large extent, the final outcome of a radiation exposure of the developing brain.

62. It has been confirmed that the period of maximum sensitivity of the brain structures corresponds to the period of maximum activity in neural cell production between about 8 and 16 weeks post-fertilization in man. It is also apparent that brain histogenesis and maturation take place in various animal species at different times and that in man the maturation of the CNS extends from the embryonal well into the fetal and even the post-natal ages. This accounts for the different sensitivity of the brain structures in various species to the damaging action of radiation. Also, different parts of the brain develop and mature at different times, so that one should expect different periods of sensitivity for the functions associated with each of these structures. In contradistinction with what happens in rodents, the development and maturation of the human brain cortex lasts relatively longer than the major organogenesis of the brain: this may explain why, in man, disturbances of higher nervous functions are relatively more common after irradiation than is gross teratological damage.

63. The following concepts, derived from what is currently known about human brain development, structure and function, should be emphasized for their importance in respect of radiation effects. First, the neurons are perennial cells: they are generated once and for all in the course of cortex histogenesis and their inactivation by the action of radiation (or any other embryotoxic agent) is likely to result in permanent loss of mental functions. Secondly, brain structures are arranged in anatomical centres related to specific functional activities; inactivation of these centres is likely to result in a deficiency of the specific functions. These functions are not recovered by repopulation of glial cells. Thirdly, the brain functions are critically dependent on the establishment of special connections between neuronal cells in various parts of the CNS. A programmed sequence of events allows the cellular architecture to be established according to precise spatial and temporal arrangements. Interference by radiation, or other agents, in such a highly structured developmental plan is likely to result in a disruption of the neuronal connections, with the related functional loss.

64. The incidence of congenital abnormalities in the human species is highly dependent on the method and the time of scoring. They are found in about 6% of new-born individuals. Very roughly, they may be classified according to their etiology into those of simple genic origin (about 6% of all malformations at birth); those having a complex multifactorial origin (about 50%); those due to chromosomal defects (about 5%); and those known to be associated with various environmental factors (around 6%). Finally, there is a large class comprising about one-third of all abnormalities visible at birth which includes all those without an apparent cause. Radiation-induced malformations must be viewed against this background of naturally occurring conditions.

65. Children seen to be malformed at birth are only the survivors of a much greater number of malformed embryos or fetuses that have died at some stage in the intra-uterine development. If scoring is done on grown-up children the incidence of malformed ones is higher than at birth. Incidence figures are highly dependent on the method of scoring, on the size of the sample and on diagnostic criteria. The ethnic group, the familial genetic background, the age, parity and social class of the parents, the geographic location, the season of the year are also important sources of variability for malformation incidence. For the purpose of this study it is assumed that approximately 6% of all new-born children are affected by structural

abnormalities that are sufficiently severe to disturb their physical well-being and in some cases to decrease their viability.

II. THE PRE-IMPLANTATION PERIOD

66. It is very difficult to study, in man, events taking place in the conceptus before implantation. This is because there is no way of knowing whether fertilization has taken place, until the most sensitive radioimmunoassay tests show an increased concentration of human chorionic gonadotropin (HCG) in urine, thus indicating trophoblastic activity. It is widely believed, however, that many pregnancies end in embryonic losses before pregnancy has been clinically diagnosed and often before the first missed menstruation [G16, K39, M7]. These considerations explain why direct human observations of the pre-implantation stages are very rare for both normal development or after embryotoxic treatments. Of necessity, therefore, one must rely on observations in experimental animal systems. The short duration of the pre-implantation phase relative to the total duration of pregnancy in man, and the high rate of embryonic loss during this phase, must be kept in mind in order to assess appropriately any radiation effects that may be induced during this period of development. The scope and the limitations of model systems in teratology have been discussed in a paper by Beck [B29].

67. From its previous analysis, based exclusively on animal data, UNSCEAR concluded [U2] that death of the embryo was the most conspicuous effect to be seen after irradiation in the pre-implantation phase. Reduction of body growth and induction of malformations were not reported after irradiation at this stage of development. Chromosomal damage in the irradiated blastomeres, leading to degeneration of the primitive cells, is the major mechanism responsible for the killing of the embryo. Substantial differences in sensitivity were noted depending on the various species and as a function of time from fertilization in the early segmentation stages. In the mouse, the rodent species to which most data referred, data in good agreement pointed to a risk coefficient for killing of zygotes soon after fertilization, of the order of 1 Gy^{-1}. The following text updates these conclusions by review of the new findings on in vitro and in vivo systems.

A. EXPERIMENTS IN VITRO

68. Since the 1977 UNSCEAR report [U2], the culture in vitro of fertilized mammalian oocytes up to defined stages in their development has been widely used in many laboratories, both as a means to investigate mechanisms of damage in the pre-implantation stage and as a useful test of effects induced by radiation when administered alone or in combination with other embryotoxic agents. The main advantages of this technique are: incubation under well-defined conditions; careful timing of the treatments; good plating efficiency and capacity for further differentiation; and possibility to reimplant the blastocysts into foster mothers to follow their further development [J15, S19]. The reports dealing with mechanisms will be examined here, while the work in which in vitro culture of embryos has been used simply as a test for embryotoxicity will be reviewed in VI.B.

69. Alexandre [A3] irradiated 2-cell or morulae mouse embryos (x rays, doses from 0.5 to 10 Gy) and cultured them in order to follow the percentage of embryos that would reach the blastocyst stage at various times post-irradiation. The most significant effect for irradiation of 2-cell embryos was the inhibition of primary differentiation shown by the failure of the embryo to proceed to the blastocyst stage. No embryo reached such a stage after a dose of 4 Gy. X rays administered to advanced morulae did not inhibit primary differentiation, although they did affect, eventually, the hatchability of the blastocysts. In a subsequent paper [A4], the same author showed that the reduced capacity to reach the stage of blastula after irradiation at the 2-cell stage was the result of early killing of cells. In fact, blastocysts from irradiated embryos contained a smaller number of cells. A lack of inner cell mass and its derivatives in further development was the end-effect of irradiation for both the 2-cell and morula-stage embryos.

70. Two papers by Pedersen et al. [P1, P2] showed that early mammalian embryos possess the enzymes required to perform excision repair of DNA damaged by ultraviolet (UV) light. Embryo cells maintained a relatively constant level of excision repair capacity until the late fetal stages, when such capacity was lost. However, there seemed to be no simple relationship between these changes in repair capacity and the known changes in the radiation sensitivity of embryos and fetuses.

71. In a small series of experiments, Jacquet et al. [J1] followed, as a function of time after fertilization, the development in vitro of cultured mouse embryos that had been irradiated with a series of x-ray doses (from 0.25 to 1 Gy) 2.3 or 6.7 hours after induced ovulation. The authors concluded that the sensitivity of the mouse egg was high at the pronuclear stage before DNA synthesis began, when doses of 0.2 Gy were sufficient to increase pre-implantation loss. These results in vitro parallel earlier in vivo findings by Russell and Montgomery [R20] who reported that the sensitivity to radiation-induced killing and to chromosome loss was very high shortly after sperm entry, and again at the early pronuclear stage, but became low in a late pronuclear stage. Jacquet's findings [J1] are at variance, however, with those of Schlesinger [S4] who found no difference in the radiosensitivity at any time during the first day after fertilization.

72. Jacquet et al. [J10] also reported on a preliminary study of the synthesis of DNA in mouse zygotes. They irradiated, with a range of x-ray doses (0.25 to 1.0 Gy), superovulated mice at the time of fertilization, at the beginning and at the maximum of DNA synthesis, and at the beginning of the first cleavage metaphase. On the day following irradiation the embryos were collected and classified as either uncleft

or 2-cell embryos; the latter were followed for a further 7 days. The pronuclear stage was found to be the most sensitive: irradiation at this time resulted in high mortality prior to first cleavage and at the 8-cell (1 Gy) or morula (0.5, 0.25 Gy) stages. Hatching of the blastocysts and attachment to the bottom of the culture flasks with the formation of a normal inner cell mass (which phenomena are referred to by the authors as "implantation") were also impaired. Cytogenetic studies on metaphase chromosomes also confirmed the well-established notion [U2] that embryonic death is attributable to chromosomal damage.

73. Domon [D10] analysed the cell-cycle-dependent radiosensitivity of BDF_1 mouse 2-cell embryos. The cell cycle of these embryos lasts 18 hours and is characterized by a long $G_2 + M$ of 14 hours and an S phase of 4 hours, a figure that is shorter than in other cases [M47, S70]. The author followed, as a function of time, the changes of the dose needed to prevent 50% of the cells from reaching the blastocyst stage (LD_{50}) and found that the LD_{50} varied within a factor of about 6. The LD_{50} of the embryos was about 2 Gy during the S phase and increased sharply in early G_2 ($\sim$ 6 Gy); resistance gradually dropped (to $\sim$ 1 Gy) as they progressed to M. Domon concluded that the position in the cell division cycle at the time of irradiation is a major determinant for the in vitro response of the early embryo to irradiation. The range of variation of the radiosensitivity values observed within the cell cycle appears, indeed, much larger than the range of radiosensitivities of pre-implantation embryos in different developmental stages (see Table 4, annex J, in [U2]). One-cell-stage mouse embryos were also found to be more sensitive than two-cell stages in experiments by Ku and Voytek [K18].

74. Domon [D11] also measured the dose required to prevent development of 50% of the embryos to blastocysts during 5 days of culture in vitro of pronuclear mouse embryos irradiated at various times during the cell cycle. This dose was found to vary from 1 to 2 Gy during the time from G_1 to the first cleavage. Pronuclear embryos were found to be more sensitive to radiation than embryos in the 2-cell stage [D10]. However, when radiosensitivity was related to the number of blastomeres, the pronuclear embryos appeared to be as sensitive as the 2-cell ones. It was concluded that the proportion of cells of various cell-cycle ages in the conceptus governed radiosensitivity during the early cleavage stages.

75. Yamada et al. [Y3] addressed the problem of the rapid changes in radiosensitivity, observed in the fertilized ovum during the time from fertilization to first cleavage, by the use of a mouse in vitro fertilization system. They found that the LD_{50}, as defined by Domon [D15], varied rapidly and extensively over a range of about 40 to 400 R. Sensitivity was relatively low soon after sperm entry and became progressively high (40 R) 4 to 6 hours after insemination, just before pronuclear formation. In the later pronuclear stage (12 hours after fertilization) sensitivity became low (400 R), only to rise again just before first cleavage.

76. In another paper by the same group [M48], mature sperm, eggs just before insemination and zygotes in the early pronuclear stage were exposed to x rays (40 R) and then cultured in vitro. The control non-irradiated zygotes, and those derived from irradiated sperm and eggs, progressed to the metaphase stage in over 90% of the cases by 15-17 hours from insemination. By this time, however, about 40% of the 1-cell eggs were still at the pronuclear stage, following irradiation in the early pronuclear phase. There was often a delay of chromatin condensation in one of the two pronuclei. In agreement with these observations, the incidence of chromosomal aberrations in first-cleavage metaphases of zygotes was much higher (20%) when irradiation took place on the zygotes than when it was carried out in sperm (2.9%) or unfertilized eggs (11.0%).

77. A series of papers by a group in the Federal Republic of Germany dealt with mechanisms of radiation damage during the initial phases of mammalian embryonic development. One of these papers [M11] showed that pre-implantation mouse eggs irradiated with x rays or neutrons in the G_2 phase of the 2-cell stage contained micronuclei and chromosomal aberrations when they reached the 4- and 8-cell stages. Micronuclei were more numerous in the 8-cell embryos, while chromosomal aberrations (particularly of the chromatid type) appeared more numerous in the 4-cell stage. As micronuclei derive from acentric fragments following chromosomal aberrations, the sequence of events would thus be logically explained.

78. Other papers [M32, M34] described the cell kinetic events following irradiation of the G_2 phase of the 2-cell mouse embryo. Doses of x rays between 0.12 and 1.88 Gy were delivered to such embryos, and their development up to blastocysts was followed as a function of time. A dose-related impaired hatching of blastocysts, and a reduced number of cells per blastocyst, were observed. Recovery and survival of irradiated embryos was also found. Moreover, normal, healthy mice could be produced by blastocyst transfer into foster mothers even after 0.94 and 1.88 Gy. A radiation-induced G_2 block of the cells, leading to a disturbance of cell progression along the cycle, was described. Hypodiploid cells were seen in later cell cycles, presumably related to the loss of genetic material induced by chromosomal aberrations. A finer analysis of the radiation-induced G_2 block after irradiation of embryos in various stages of development and of the resulting effects was also published by the same group [M12].

79. The DNA amounts and cell cycle phases were studied in mouse embryos after administering a dose of 1.88 Gy to 1- or 2-cell mouse embryos [M13]. Blastocyst formation was more impaired (28% of control) following irradiation of the 1-cell than of the 2-cell embryos (73% of controls) and cell death was mostly responsible for the impairment of the embryonic development. Cell death was well correlated with the presence of micronuclei after irradiation, and both phenomena were most evident after irradiation of the 1-cell zygote than of the 2-cell embryos.

80. The cell proliferation in vivo and in vitro of pre-implanted mouse embryos was investigated from 2 to 144 hours after conception [S70] by measuring the number of cell nuclei, the DNA content of the cells and the mitotic and labelling indices. After the first cell cycle the length of the S phase remained constant at about 7 hours, while G_1 and G_2 varied considerably. The increase in the number of cells was exponential from 31 to 72 hours after fertilization, but the rate of growth during this time was slower in vitro than in vivo. Also, the proliferation rate slowed down as the development proceeded to blastocyst. Although, in general, development was judged to be slightly faster in vivo, the phenomena were found to be very comparable under both conditions: this is evidence that the technique of in vitro culture is suitable for studying early kinetic events in the developing embryo.

81. Molls et al. [M47] compared, by using cytofluorometric techniques, the progression through the first few cell cycles of pre-implantation mouse embryos of the Heiligenberger and NMRI strains. Ova fertilized in vivo during a short mating period were followed up to about 4 days from conception and the duration of the cell cycle phases was estimated at various times directly from the DNA histograms. Differences between the two strains were mainly in the length of the $G_2 + M$ phases. In both strains, the length of S increased from the first to the second cell cycle and the G_1 periods of the second and third cycle were very short. The increase in total cell number was exponential by the third cycle, with some differences in the rate of proliferation between the two strains. By the end of the pre-implantation development, when embryos reached the stage of hatched blastocysts, growth started to decline. The differences found were considered to be strain-specific and not due to the methods used for DNA measurements or to the different degree of synchrony of the embryos in the two strains.

82. In experiments on the preservation of mouse stocks by low-temperature storage of early embryos, Whittingham et al. [W11] kept 8-cell embryos in liquid nitrogen at −196° C. They were exposed to various levels of background radiation ranging from 1.8 to 84 times the "normal" background, for periods ranging from 6 to 29 months. The capacity of these embryos to reach the blastocyst stage in vitro or to reach fetal or live-born stages when transferred to recipient females were the end-points used to assess the effects. Comparisons with non-frozen or short-term frozen controls were also made. The low-temperature treatment itself was responsible for a marked loss of viability, so that when all data were pooled, only about 20-30% of the embryos originally frozen were recovered as fetuses or live-borns. By comparison with this high rate of loss, the effect of background was absent or non-significant. Only the embryos kept for 29 months at 84 times the control background showed a slight reduction of implanted and live fetuses. However, even this effect was of doubtful significance in view of the lack of any relationship with the time of storage.

83. When assessing the usefulness of techniques of assay in vitro for exposure of embryos in vivo, direct comparisons by the evaluation of similar end-points are of importance. In this respect, it is appropriate to cite a paper [F2] which escaped the previous UNSCEAR review [U2]. In these experiments, 2-day-old mouse embryos of the 129/SvSl strain were obtained by hormonally-induced superovulation. They were treated with increasing exposures from 220-kVp x rays (from 6 to 1455 R) either in vitro or directly in the superovulated females. Embryos were then removed from these females within 2 hours and cultured in vitro up to blastulation under conditions similar to those used for the embryos irradiated in vitro. Blastulation occurred in about 80% of cultured sham-irradiated control embryos. It was found, in essence, that under all conditions the inhibition of blastocyst development by irradiation was dose-related. However, the exposure required to inhibit blastulation of 50% of the embryos was about 300 R for irradiation in vitro and about 600 R for irradiation in vivo.

84. After 48 hours of culture, embryos that had blastulated were again transplanted into pseudo-pregnant females which were killed 15 days later for scoring of implantation scars, resorptions and fetuses (weight, gross abnormalities, skeletal abnormalities). Approximately 70% of implanting control blastocysts developed into fetuses. After in vitro exposures in excess of 73 R, significantly fewer fetuses developed. Exposures of the order of 170 R resulted in no fetal development at all, and those of about 270 R resulted in no implantation. Following in vivo irradiation, fetal development occurred up to 388 R. No more or no different gross and skeletal abnormalities were observed significantly above the control level after either in vitro or in vivo exposure. As to weight changes, an impairment occurred in fetuses developing from transplanted embryos irradiated in vitro with 73 R or more. It was concluded that the lower susceptibility to radiation damage found for embryos irradiated in vivo was due either to direct embryo shielding or to a protective physical or metabolic mechanism provided by the maternal tissues and operating within the 2-hour interval between irradiation and transfer to culture. However, the details given in the paper [F2] about radiation dosimetry and the use of exposures, rather than absorbed doses, render any quantitative assessment of these data rather problematic.

B. EXPERIMENTS IN VIVO

85. By comparison with reports on irradiation in vitro, the reports on in vivo treatment of pre-implantation stages are few. In a series of papers on the effects of gamma rays and helium ions on embryonic rats (0.5-6.0 Gy), Ward et al. [W1] exposed pregnant animals on one of days 4 to 9 of gestation (the pre-implantation stage ends in the rat at about day 7) and scored the embryo survival at 20 days p.c., as a function of the embryo position in the uterus. They found that embryos located in the ovarian or in the cervical ends of the uterus had higher rates of mortality than their litter-mates implanted in the middle part of the uterine horns. This conclusion was true for both radiations and for all irradiation times. The influence on survival of the uterine position

increased with the dose of radiation and with the number of implants, so that with high doses and a crowded uterus the probability of survival of embryos in the least advantageous positions could be as low as one-half of that of the litter as a whole. Irradiation under hypoxic conditions also led to a high rate of killing of embryos implanted in the cervical end of the uterine horns: this suggested that the selective distribution of intra-uterine deaths could not be attributed to a state of relative hypoxia. The non-random distribution of the killing effect was in contrast with the random occurrence of the gross morphological abnormalities to be seen in the irradiated litters.

86. In another experiment [S4], pregnant mice were exposed to 100 R of x rays at various hours in the first day of gestation and the fetuses were scored at term for death during the pre-implantation or early post-implantation stages. An increased rate of both types of death was induced by the treatment, accompanied by an increase in the mean fetal weight and a decreased incidence of malformations. Changing the irradiation time did not affect survival of the animals at term. At various times after irradiation, the embryos were tested by the eosin Y supravital staining method to assess cell viability. The relative frequency of viable and dead cells in normal or irradiated embryos was found to be comparable at 1, 2 and 3 days. At 4 days of gestation, an increased proportion of dead cells was noted in both the control and irradiated embryos: this was taken as evidence of the occurrence of cell death as a normal mechanism in the course of mammalian development. At this time, irradiation caused a decrease in the percentage of embryos reaching the blastocyst stage and a decrease in the number of cells per embryo. The authors noted that the lack of a significant increase in the percentage of dead cells in irradiated mice could be due to the high variability of the findings masking the killing effect of radiation or to the fact that cell death may become manifest only after the fourth day of gestation. This research is broadly in agreement with many other experiments [U2] confirming that embryonic death is the main effect found after pre-implantation irradiation. However, the single exposure used, the short time tested, the lack of a precise timing for mating, and the non-specificity of the techniques used, render these conclusions of rather limited significance.

87. Roux et al. [R21] investigated the effect of 0.05, 0.1, and 0.25 Gy of gamma radiation administered in single doses to Wistar rats 9 or 16 hours after mating. At 9 hours, DNA synthesis had not yet begun, and at 16 hours was still taking place in the ovum. The pregnant females were killed on the 21st day of gestation for counting of the total number of implantation sites, the number of live fetuses, resorption sites (embryonic death between 7 and 12 days) and dead fetuses (death after day 12). Live fetuses were also weighed and examined for malformations of the head and skeleton. The pre-implantation embryonic mortality, the mean weight of the fetuses and the sex ratio were not significantly changed by the radiation treatment. There were also no differences in the type and incidence of malformations in the various experimental groups. However, mortality (both embryonic and fetal) was increased by the radiation exposure, without any significant difference between the two irradiation times. At 0.05 Gy the increased mortality only occurred at the fetal stage; at 0.1 Gy both the embryonic and fetal mortality were enhanced, but the latter more than the first: at 0.25 Gy the stage of conceptus at death depended on irradiation time, because embryonic mortality was always increased, although much more in the 16-hour group, while fetal mortality was only increased in the 9-hour group. These results are in agreement with previous ones in showing that mortality, rather than malformation induction, is the prevalent effect of irradiation during the pre-implantation stage. They are, however, at variance with the previously discussed notion [U2] that pre- and early post-implantation death is generally seen under such conditions of exposure. As to the dependence of mortality on irradiation time, the present series [R21] shows a complex relationship between dose and time at irradiation.

88. Aldeen and Konermann [A2] treated pregnant mice by a single exposure of 300 R x rays 1, 2 and 3 days p.c. and dissected the uteri 6 days p.c. The mean number of implantation sites was found to be 9.67 per female in the controls and 8.00, 6.63 and 7.00 per female in animals irradiated 1, 2 and 3 days p.c., respectively. Among 22 implants that were examined histologically after irradiation on day 1 p.c., no living embryo could be detected: the same was true of 19 implants on day 2 and 11 implants on day 3 after irradiation. The percentage of resorptions was lowest on day 1 after irradiation (31.8%) and highest (94.7%) on day 2. Cytological and histochemical criteria were used to assess the state of the decidua, which was either normal or only marginally affected on day 2 after irradiation, concomitantly with the peak of embryonic deaths. From this the authors concluded that direct embryonic killing, and not maternal effects, were involved in the damage described.

89. Zhou and Wang [Z3] submitted mouse embryos in the pre-implantation period to single or fractionated exposures of radiation (gamma rays, 100 R). Among early effects, they reported an increased mortality (as evidenced by an increased number of dead fetuses and resorption sites) and a decreased mean fetal weight of irradiated litters.

C. CONCLUSIONS

90. The latest information on the effects of embryo irradiation during the pre-implantation stages does not include data for man. In the last few years, techniques of embryo culturing in vitro have been widely employed in experimental animals to study a variety of problems, mostly dealing with the mechanisms of radiation damage during this stage. For such problems, in vitro culture offers significant technical advantages and results that are qualitatively comparable with those obtainable in vivo. The similarity of the findings between in vitro and in vivo analyses does not extend, however, to the quantitative aspects of

radiation damage: conclusions drawn in vitro may not easily be extrapolated to in vivo irradiation. Another condition that impedes generalizations across species is that the latest data refer only to mice and rats.

91. Experiments in vitro have made it possible to ascertain that the sensitivity of the embryos is highest for irradiation of the pronuclear stage. They show quite conclusively that there are oscillations in the response of the embryos related to the cell division cycle. Doses as low as 0.2 Gy have been reported to cause statistically significant embryo killing at the time of maximum sensitivity before DNA synthesis begins. However, the variability of doses required to kill one-half of the cultured embryos (LD_{50}) spans a factor of about 10 among different phases in the mitotic cycle and different mitotic cycles. The formation of blastocysts is more damaged when irradiation takes place at the 1-cell than at the 2-cell stage, and there is a good correlation between the death of the embryo after irradiation and the occurrence in it of chromosomal abnormalities. Irradiation also disturbs the kinetics of cell progression, mostly through a variation of the duration of G_1 and G_2 phases.

92. Irradiation of the 2-cell embryo results in a dose-related block to proceed to the blastocyst stage. Doses of a few Gy are needed to stop completely 50% blastocyst formation. As the embryo maintains a reasonable degree of synchrony during the first few segmentation divisions, its sensitivity may be analysed as a function of cell cycle phases. Under these conditions, the changes of embryo sensitivity result in LD_{50} values ranging from 1 to 2 Gy (irradiation in M and S) and 6 Gy (irradiation in G_2). A G_2 block of the cell cycle has also been described for irradiation at this stage. Hatching of blastocysts is, in general, a more sensitive end-point. Cell killing by induction of chromosomal aberrations appears to be the cause of a decreased rate of growth of the irradiated embryo and, ultimately, of the embryonic mortality. As the number of cells in the embryo increases, the dose needed to stop embryonic development to a given stage appears also to increase. However, for irradiation at a given stage, the apparent radiosensitivity of the embryo to killing increases with the time intervening between irradiation and the scoring of the effect.

93. Experiments in vitro have documented the existence of some strain-related variability in the rate of progression of the embryo through the initial developmental stages in the mouse. There may also be differences in the intrinsic radiosensitivity of early mouse embryos of the same strain irradiated in vitro or in vivo, the latter condition being apparently less damaging. Transplantation into foster-mothers of embryos irradiated and cultured in vitro allows the surviving embryos to complete their development to normal animals. Such experiments have confirmed that loss of viability is the only effect to be seen for treatment during the pre-implantation stages at the two-cell stage or later. Weight changes in the survivors could be secondary to the killing of some of the conceptuses in polytocous animals. Radiation-induced teratological effects in excess of the natural level have not been documented.

94. Experiments on rodent embryos irradiated in vivo have confirmed the above in vitro conclusions, at least qualitatively. Embryonic killing emerges in vivo as the major effect of irradiation. It appears to be due to an action on the embryos that is direct and not mediated through disturbances of the formation of the placenta. In vivo, relatively small doses (0.05 Gy) have been reported to result only in fetal mortality, but after higher doses (0.1 Gy) embryonic mortality is also affected. Oscillations of embryonic radiosensitivity would appear, on the whole, to be less prominent for irradiation in vivo: this could, however, be due to the lesser degree of synchrony that obtains under these conditions.

III. THE PERIOD OF MAJOR ORGANOGENESIS

95. In its 1977 review [U2] UNSCEAR concluded that the most characteristic (but by no means the only) type of damage to be seen in mammalian embryos irradiated during the period of major organogenesis was the induction of malformations. The review discussed the conditions under which teratological damage became apparent and stated that, at least qualitatively, similar classes of malformations occurred in various animal species upon irradiation of comparable developmental stages. Inter- and intra-species variability was also reviewed on the basis of the data then available. The review discussed the presence and timing of the periods of maximum sensitivity for certain classes of malformations and observed that these maxima usually coincided with the major phase of morphogenesis of the relevant anatomical structures. It observed that, although the dose-response relationships for a given type of malformation were of the sigmoid type, grouping of embryologically or topographically related malformations or expressing the teratological damage as the ratio of malformed to normal animals, irrespective of the type and degree of malformations carried, tended to generate quasi-linear dose-effect relationships. Data then available concerning rodents (the projection of which to man would, however, be unwarranted) tended to yield estimates of the absolute increase of incidence of malformed fetuses per unit absorbed dose of the order of $5\ 10^{-1}\ Gy^{-1}$ of low-LET radiation delivered at high dose rate. In man, an estimate of incidence of severe mental retardation per unit absorbed dose was tentatively set at $10^{-1}\ Gy^{-1}$, based on the data from Hiroshima and Nagasaki. Other epidemiological surveys in man, after doses in the region of 0.01-2.0 Gy, were only of indirect value in excluding the possibility that at such doses the human embryo could be 10 times more sensitive than implied by the incidence of malformations at higher doses [U2].

96. The information that has since become available has added considerably to our knowledge of phenomena induced by irradiation in experimental animals, particularly in the CNS. New data have also been produced on the occurrence of mental deficiencies in children irradiated during the explosions at Hiroshima and Nagasaki. All the relevant material on malforma-

tions of the CNS in experimental animals is being reviewed here, partly because it would be difficult and repetitive to separate out from the various series data referring to either embryonic or fetal stages; and partly because mental retardation in man (which occurs especially at times which are at the borderline between the two developmental stages) is also treated here. It is recognized, of course, that cases of mental deficiency may also appear in children irradiated at times which are considered well within the fetal stage of development. However, by discussing all data together it is hoped to convey a more coherent picture of the malformations observed and of their incidence as a function of dose and time. The reader is referred to chapter I for a brief description of normal CNS development in animals and man.

A. DATA FROM ANIMALS

1. Skeletal malformations

97. Single exposures from ^{60}Co radiation, in the range from 50 to 500 R, were given to about 600 pregnant Swiss mice at 9, 10, 11 and 12 days of pregnancy [P3]. Starting from day 13, and up to day 18 p.c., the embryos and their placentas were weighed and examined. For irradiation on day 9, exposures below 150 R produced no significant deviations from controls, whereas higher exposures were lethal to the conceptus. For irradiation on day 10, an exposure of 100 R was the threshold for effects on the weight of the placenta. Malformations became apparent at 125 R. At 150-175 R, fetal mortality was over 60% and surviving fetuses were malformed. At 200 R, fetal loss approached 100%. On day 11 the feto-placental complex was reduced in weight at 75 R. At 200 R, prenatal death was over 60%, and at 300 R it was close to 100%. On day 12 the weight of the conceptus and placenta declined between 125 and 450 R. Malformations were seen at 150 R, and 500 R produced total loss of the fetuses. The data were compounded into an overall model of action accounting for dose, time and type of effects.

98. Tribukait and Cekan [T3] carried out experiments on C3H mice, primarily to study the form of dose-effect relationships for malformations brought about by irradiation with single, fractionated or protracted doses during the period of major organogenesis. Pregnant females irradiated at 9 days p.c. with 180- or 250-kV x rays were killed at 18 days p.c. for examination of implantations, resorptions, living and dead fetuses and fetuses with gross external abnormalities. Skeletal and visceral malformations were also specifically studied, but the incidence of this latter class was too low and its scoring was later omitted. Single doses in the range of 0.125 to 2.5 Gy were delivered. In total, 50 malformed out of 1929 non-irradiated fetuses were found; 1320 malformations were seen in 3558 irradiated animals.

99. The frequency of malformations belonging to various classes was very different when all irradiated and non-irradiated animals were compared. In the vertebral column and ribs the frequencies were 32 and 44% in non-irradiated and irradiated, respectively; umbilical hernia was, respectively, 14 and 6%; digital malformations 8 and 6%; anophthalmia, and microophthalmia and other malformations of the eye 8 and 18% (although coloboma was mostly seen in the irradiated mice). Tail malformations were, in both cases, 18%. Dose-response curves for resorptions and malformations were of the sigmoid type, with a very low incidence of the effects up to 0.75 Gy and a steep rise thereafter. Resorptions, in particular, with a control incidence of 10% did not show any clear trend with dose, up to about 1 Gy. Taking the various classes of malformations separately, absolute increases of incidence per unit absorbed dose of between 9 and 54% Gy^{-1} were observed in the steep region of the curves. Pooling all malformations, a 57% increase per Gy resulted, with practically 100% malformed animals at 2 Gy. For doses below 0.75 Gy, the absolute increase in the percentage of malformed mice would be 0.1% per 0.01 Gy (Figure VIII). Expressing the effect as number of malformations per fetus produced a similar pattern, with an initial increase to 1.4 malformations per animal up to 0.75 Gy and a further, steeper rise to 3 malformations per fetus up to the highest doses used.

100. Dose-fractionation experiments were also carried out [T3] in which the embryos received a total of 1.75 Gy, split into 2 fractions delivered at intervals between 15 minutes and 8 hours. Even taking into account that a decrease in the sensitivity of the system occurred over this time (resorptions dropped from 45 to 34% and malformations from 45 to 21% following single doses of 0.875 Gy) an interval of 15 minutes brought about a 30% reduction in the incidence of both effects, followed by an increase at 4-6 hours and a further decrease to near control values at 8 hours. This pattern is reminiscent of the two-dose recovery curves commonly described for many mammalian cells and tissues. Different responses as a function of fractionation time were noted for the various classes of malformation, those of the eye being apparently more spared by dose splitting than the skeletal ones.

101. Further experiments were conducted [T3] in which the same dose of 1.75 Gy was delivered at dose rates of 0.72, 0.055 and 0.015 Gy min^{-1}. The total incidence of malformations increased from 55 to 80% between the highest and the lowest dose rates tested, but different malformation types were differently affected, the external and the ocular malformations being practically unchanged and the skeletal ones essentially doubled. This finding is at variance with the majority of data discussed in the 1977 report [U2], but it is consistent with the notion that dose-rate changes and fractionation may affect various malformations differently. Cell kinetic data obtained by whole embryo preparations were also given in [T3], but the significance of such findings in relation to the problems at hand is not clear.

102. A thesis by Knauss [K8] contains accurate data on the dose-response relationships for mouse skeletal malformations induced by pre-natal exposure to radiation. Mice of the Heiligenberger strain were exposed at 7, 10 and 13 days p.c. to 150-kV x rays in the range of

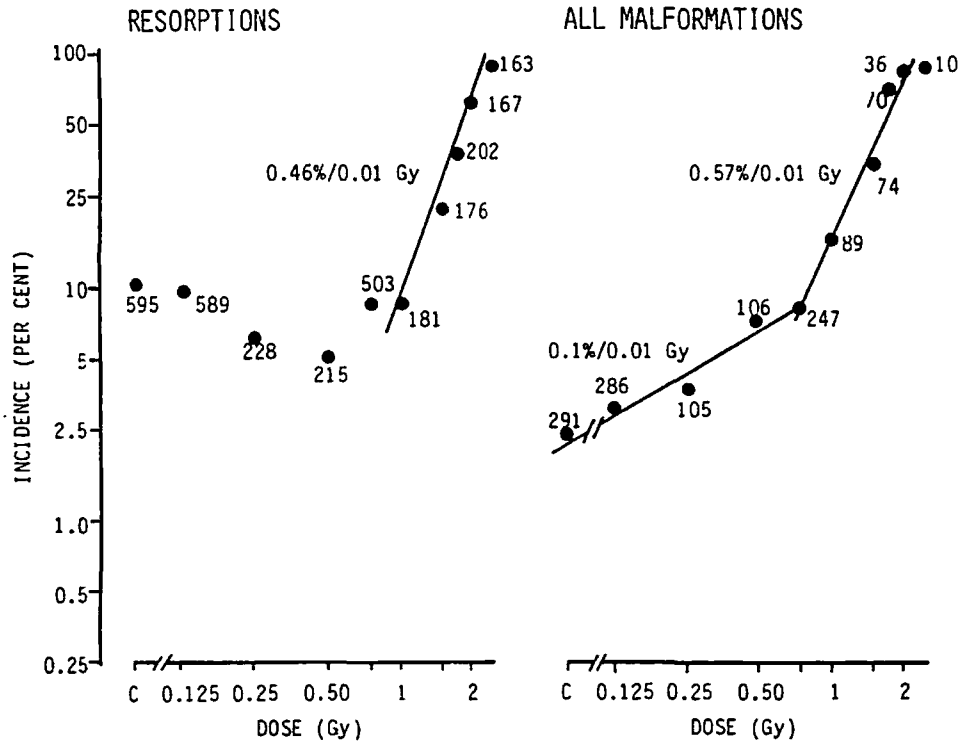

Figure VIII. Dose-response relationships on a log-log scale for resorption (left) and all malformations (right) incidence in mice irradiated at 9 days p.c. The number of observations is given for each experimental group.
[T3]

5-250 R and examined on day 19 p.c. for the presence of minor variants, abnormalities or major malformations of the skeleton, after staining with Alizarin S. The various types of effect were examined separately or jointly as a function of dose. The 7th day p.c. was the time at which the sensitivity appeared to be at its maximum; the minimum was at 13 days p.c. By considering each class of malformation (they were divided according to the various sections of the skeleton), quite pronounced threshold-type exposure-incidence curves were obtained. The data were also treated in such a way as to obtain estimates of fetuses without abnormalities or malformations (excluding the variants). They were plotted, as a function of dose, as in Figure IX. That figure shows that exposures of the order of about 80 or 140 R would be needed to induce malformations in 50% of the irradiated fetuses at 7 or 10 days, respectively. The difference is due mainly to the wider threshold observed at 10 days p.c. These experiments are to be regarded as a further confirmation of the non-linearity of the dose-response relationship for the induction of malformations at the most sensitive stages in development.

103. These and other experiments were later included in a review by Konermann [K12] from which Figure X is taken. The experimental points in this figure are based on 500 to 800 mice each. By plotting the incidence of gross skeletal malformations as a function of x-ray exposure on a semi-logarithmic graph, it is shown that the spontaneous incidence of 0.6% malformations is increased by a factor of 3 after an exposure of 5 R on day 7 p.c. The increase is due essentially to the contribution of malformations in the skull and the cervical column. The decrease in incidence from 5 to 25 R is attributed to the confounding influence of embryonic selection. An exposure of 25 R on day 10 p.c. doubles the incidence of malformations seen in the control mice, which is, however, unaffected by an exposure of only 12.5 R.

104. In the course of work on sensitivity analysis for an in vivo screening test for teratogens, Russell [R19] explored the possibility of using homeotic shifts in the quantitative configuration of the axial skeleton. Having ascertained that about 9 days p.c. was the optimum time for induction of changes in certain quantitative features of the axial skeleton of BALB/c mice, she irradiated pregnant mice at this particular stage (12.5, 25, 50 and 100 R). Fetuses were removed just before delivery and their axial skeleton was analysed. This inbred mouse strain shows a high degree of variability for several quantitative characters such as the numbers of ribs, costo-sternal junctions, pre-sacral vertebrae and sternebrae. Even the lowest exposure tested caused clearly discernible shifts in 3 of the 4 quantitative characters mentioned. By contrast, the morphological abnormalities of the axial skeleton showed much higher thresholds: of 10 abnormalities scored, none was induced at 12.5 R, and 1, 5 and 10 were induced at 15, 50 and 100 R, respectively.

2. Malformations of the eye

105. Cellular changes in the developing eye of embryonic mice irradiated on day 8 p.c. with 0.9 Gy of x rays were described by Balla and Michel [B1, B3]. The end-points scored on days 9 and 10 p.c. included eye size, mitotic activity, cell death and chromosomal

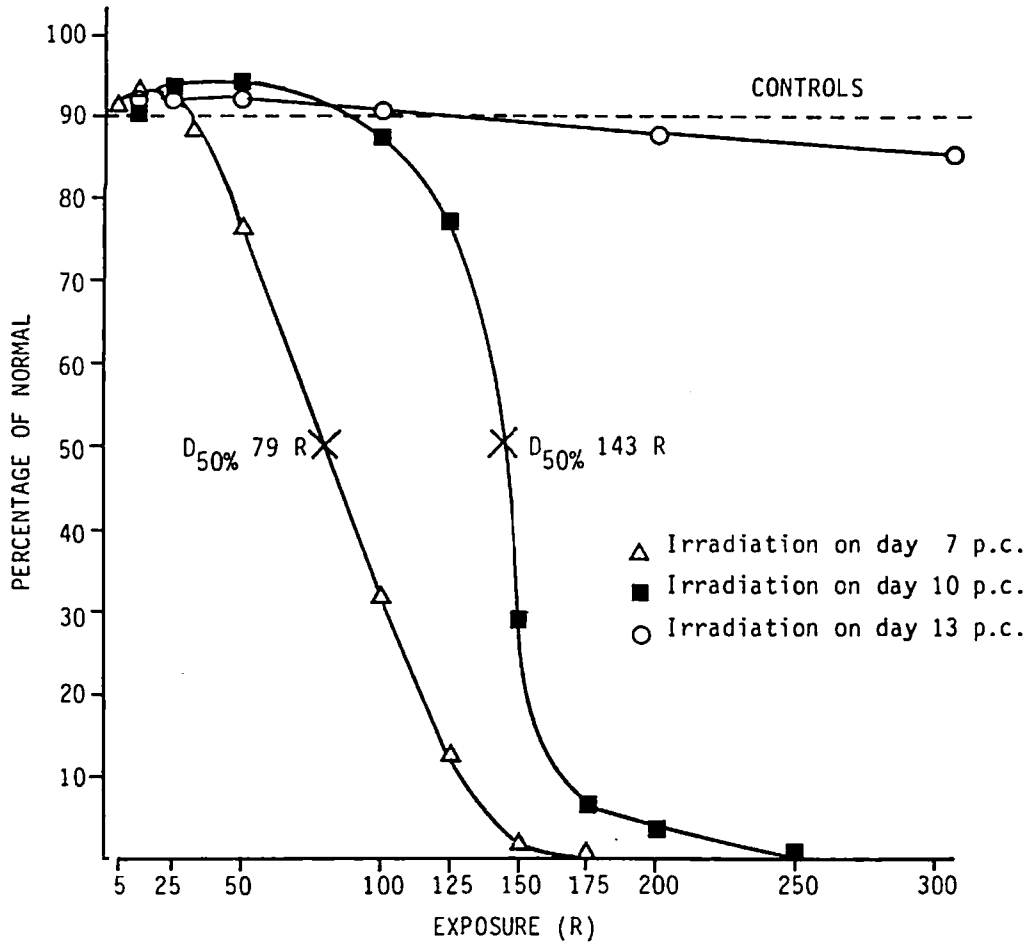

Figure IX. Percentage frequency of fetuses with normal skeletal structure, including variants, after x irradiation on days 7, 10 or 13 p.c., expressed in per cent of the total number of living fetuses on day 19 p.c.
[K12]

aberrations. The size of the eye vesicle was significantly reduced in the irradiated embryos, but was restored 24 hours later, except for a significant reduction of the lens size. In agreement with these findings, the total number of cells and the mitotic activity of the vesicle were decreased at the time of observation. The incidence of chromosomal aberrations in anaphase cells was increased in the irradiated eyes, as was the incidence of morphologically assessed cell death. Similar experiments were conducted on embryos at the 9th day of gestation. Cell death was scored at various times post-irradiation, up to 24 hours, and in different regions of the developing eye. The pattern of necrosis differed from that seen, for irradiation on day 8, and the rapid recovery occurring by 12 hours pointed to a high capacity for repair of this tissue [B2].

3. Malformations of the CNS

106. Although normal events in the development of the human nervous system have been mapped to a reasonable degree of precision (see chapter I), data on the effects of radiation directly on human material are virtually impossible to obtain. Thus, our knowledge of the pathogenesis of radiation-induced changes relies entirely on observations in experimental animals, mostly rodents. It may reasonably be assumed that, in their main aspects and for their qualitative features, the effects observed on such animals are similar to those produced in man. Hicks and D'Amato [H15], in a review of the lethal and non-lethal cell changes of the nervous system, have attempted to show the good correspondence existing in respect of the underlying mechanisms between the laboratory animal and the human data. Brizzee et al. [B9] also published a brief review of data on the structural nervous system effects caused by irradiation in utero in both animals and man. In the following paragraphs, new data on this subject will be reviewed, with special reference to those appearing since about 1977. Further data on brain damage by chronically ingested radionuclides, particularly by tritium [V2, Z1, Z2], are discussed in chapter V.

(a) The mouse

107. Kameyama et al. [K3] studied the effect of 25 or 100 R x radiation on CF-1 mouse embryos exposed at 10, 13 or 15 days of gestation, in an attempt to obtain quantitative evidence of early effects on the undifferentiated matrix cells of the developing telencephalon. Of all gestational ages, the 13-day-old embryos were found to be the most sensitive in terms of lengthening of the cell-cycle time in thymidine-labelling experiments. An exposure-effect relationship between 10 and

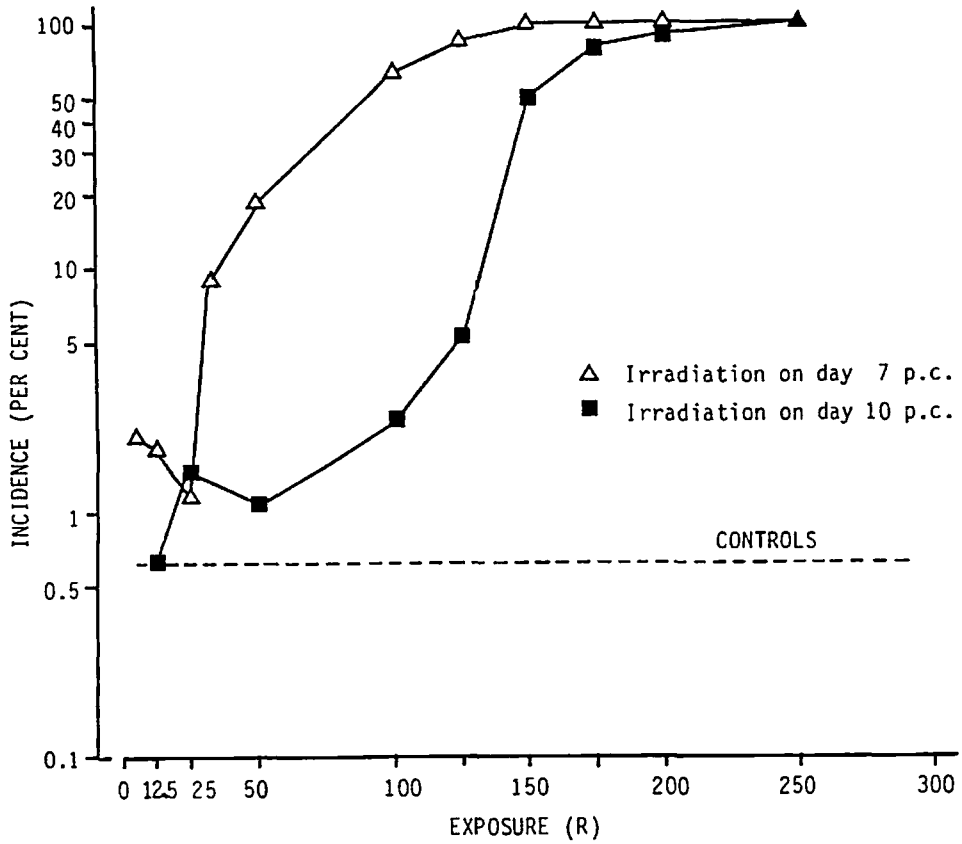

Figure X. The incidence of gross malformations in the mouse skeleton after various exposures on days 7 or 10 p.c.
[K12]

150 R was also established at this age for mitotic delay (G_2 + 1/2M) of the matrix cells. The delay was found to be directly related to the logarithm of exposure. Extrapolation of this function to the lowest exposure suggested that the apparent threshold for the effect would be slightly lower than 10 R, thus confirming the detectability of cell kinetic disturbances (the overall significance of which is, however, difficult to assess) at this exposure level. Other data on the cell cycle and radiosensitivity of matrix cells in the brain of the mouse fetuses at 13 days p.c. were also reported in an abstract by Hoshino and Kameyama [H28].

108. Hayashi et al. [H2, H4, but particularly H9] described pathological changes observable by light and electron microscopy in the telencephalon of CF-1 fetal mice exposed to 25 R or 100 R on days 10, 13 and 15 p.c. The detectability and incidence of cells with cytoplasmic inclusions by light microscopy was increased when earlier developmental stages were exposed. By electron microscopy, such inclusions could be divided into cytoplasmic degeneration and phagocytosed neuronal cells; they preceded the appearance of nuclear changes in the irradiated cells. Precise times for the occurrence of all these changes, as a function of dose and irradiation time, could be established.

109. Kameyama [K30] noted that the changes in the radiation response of the matrix cells were not apparently related with the decrease of the cells' mitotic activity as a function of time. He pointed out that the most sensitive period for these cells is when the cortical plate begins to appear. According to the author, this developmental stage in the mouse corresponds, approximately, to the 8th to 10th week of gestation in man, based on the histogenic state of the cerebral cortex.

110. Working on two different mouse strains (CF-1 and ICR), and with different gestation times (13 and 15 or 17 days), Hoshino et al. ([H12, H13], summarized in [H18]), studied the changes in the architecture of the cerebral cortex induced by 25 or 100 R. To do so, they mapped the position of thymidine-labelled neurons reaching various layers of the cortex by 4 weeks of extra-uterine age. They recognized the presence of specific changes in the various layers. The nature of the changes was found to depend on irradiation time, their severity on dose. In parallel experiments by Hayashi et al. [H10], where cortical thickness, cell density and dendritic arborization of pyramidal cells were observed, it was found that 25 R did not cause a decrease in the number of cortical cells, but resulted in a developmental effect on the pyramidal cells whose dendritic arborization was retarded. Ten roentgens, given at 13 days p.c. to ICR mice, reduced the branching indices marginally [H11].

111. Schmahl and his collaborators published a valuable series of papers in which the mechanisms and consequences of radiation-induced damage to the CNS were analysed. In a first contribution [S7], they reported on experiments in which NMRI mice received 2.0 Gy of x rays on day 12 p.c. and were examined on

day 18 p.c. for lesions of the CNS. Cell clusters (rosettes), presumably originating from the ependymal layer, were seen at this time, together with areas of necrosis close to the ventricular surface in the subependymal layer, which is the site of reparative cell proliferation. These lesions had morphologically typical aspects and evolved after birth into cysts, with flattening of the cortical wall, without reactive processes such as glial scarring. Cyst formation was accentuated after birth and evolved into porencephaly with communication between the ventricular lumen and the surface of the cerebral cortex. The histogenesis of these events, with special emphasis on the topographical differences in the developing brain, was described also in another paper [S40]. Abnormalities of the nervous tissues were often accompanied by malformations of the overlying skull, such as dysdifferentiation of the cartilage, formation of hyperostosis, and disturbances of the calcification processes [S58].

112. Another paper by the same group [S5] attempted an analysis of cellular mechanisms by a joint evaluation of pathological and cell-kinetic events in the mouse telencephalon at late organogenesis. A dose of 0.95 Gy killed virtually all matrix cells in G_1, G_2 and M, and 50-75% of those in the S phase, the survivors being responsible for the subsequent regeneration of the damaged tissue. Cell labelling allowed the differentiation of two different pools of cells surviving in S, which contributed in different ways to the regeneration processes of the telencephalic roof after radiation injury. A later study on similarly treated mice [S49] described the generation times, potential doubling times, growth fraction and cell cycle stages of the neuroblasts and their alterations following irradiation.

113. Heinzmann [H3] studied, in the mouse, the dysplasia of the third cerebral ventricle induced by 1.9 Gy of x rays on the 12th day of gestation. Perinatal observations and observations at 20 months of age were conducted by gross, microscopic and ultrastructural techniques. Brain reduction and ventriculocele were carefully described and the genesis of the lesions discussed in its possible patho-physiological mechanisms. This author also described [H19] gross changes and alterations in the fine structure of the choroid plexus of mice, induced by doses of 0.95 and 1.9 Gy of x rays given to the 12-day fetus. These changes were found to persist throughout post-natal life up to the age of 20 months.

114. In related studies [H25], the same author described the normal functional activity of the mouse brain, from the 13th day of gestation up to post-natal ages, by injection of tritium-labelled deoxy-D-glucose and radioactivity measurements. He showed that tritium uptake differed considerably between tissue samples taken from various regions of the brain at various developmental times. He also compared the normal pattern of glucose incorporation into the brain structures with changes induced by x irradiation (0.9 and 1.9 Gy) given on the 12th day of gestation. It is difficult, however, to correlate such biochemical findings with the morphological effects of irradiation which consist in a hydrocephalus of dose-related severity. Other data on the biochemistry of the mouse brain irradiated in utero have also been reported [V10].

115. Morphological effects (cell necrosis) in various parts of the brain 6 hours after irradiation with fast neutrons (single dose, 0.5 Gy) on C57BL/6 mice between the 13th and 18th day of gestation were reported [A15, V9]. Animals similarly irradiated on day 18 were examined 3 weeks after birth, at which time their body and brain weight was significantly decreased, for content and synthesis of DNA, RNA, histones and other proteins in brain and liver. These biochemical experiments suggested that damage to the developing brain by neutrons was associated with the inhibition of synthesis of certain histone fractions and non-histone proteins, probably resulting from a decreased capacity of amino-acylation by transfer RNA [A16]. (Timmermans et al. [T11], Tanaka et al. [T12], Van Beuningen et al. [V10] and Deroo et al. [D1] have also reported preliminary studies of various aspects of the biochemistry of the rat brain after irradiation in utero. Review of these data will only be possible after their publication in full.)

116. In experiments by Konermann [K34], mice were chronically irradiated in utero at various developmental stages (5-10, 11-15, 6-13 days p.c.) with a range of exposure rates from 40 to 80 R/day. These treatments induced compensatory proliferation reactions during the formation of the brain glia, and such reactions were more marked the more brain development was retarded [K11]. The lipid synthesis during the period of pre-myelination gliosis was also studied by spectrophotometric and histoautoradiographic analysis of the uptake of ^{3}H-mevalonic acid, ^{3}H-glucose and ^{32}P-sodium-phosphate in the brain. During the pre-myelination period, it was found that there was a very strict correlation between the lipid synthesis and the formation of glial cells. Such correlation was independent of the irradiation period and of the exposure rate and, since over-proliferation occurs at high doses, a rise of incorporation with increasing brain retardation (and therefore with dose) was documented. In various brain sections, in parallel with an increase of myelin density, a tighter packing of the oligodendrocytes responsible for brain myelination was also shown. From an overall evaluation of the findings, it was concluded that lipogenesis during pre-myelination occurs indirectly as a result of cell proliferation which leads, in the most developmentally retarded brains, to non-coordinated, over-compensatory reactions [K34].

117. Konermann and Schwald [K13] irradiated albino inbred mice at 13, 16, 18.5 and 22 days p.c. with 4 doses of x rays of between 0.5 and 3.0 Gy, and studied, by means of a microphotometric technique, the formation of the tigroid substance up to 80 days post-natally. The formation of this substance is thought to be related to, and reflected in, the maturation of the neurons. An irregular decrease with respect to controls of the tigroid density, which was dose- and stage-related, was observed in the irradiated animals. There were fluctuating responses also in

relation to the various brain regions examined (cortex, thalamus, cerebellum, hippocampus, gyrus dentatus, nucleus motorius trigemini) which were most pronounced during the critical periods of post-natal brain maturation of the various structures. The decrease of tigroid density was compensated at long term in the animals irradiated with the smallest dose, but with increasing doses the changes became either not compensated or progressive. Since the magnitude of the late responses decreased when the embryonic age at irradiation increased from 13 to 22 days p.c., the responses must either be extended through several cell generations or be induced to a lesser degree in the late pre-natal stages. In parallel with the responses of the tigroid substance during the second and third week after birth, there were also changes in the total content of RNA in the brain, which were again most pronounced for the earliest ages at irradiation. The overall meaning of these changes appears to be a retardation and stabilization of the brain maturation, but their significance remains to be fully evaluated and interpreted.

118. Treatment of mice in utero with fractionated doses between 3 × 1.05 and 3 × 1.33 Gy on gestational days 11-13 gave rise to rosette-like clusters of primitive cells resembling ependymal cells, dispersed within the brain cortex wall. These lesions appeared to be particularly abundant in the females, suggesting that the radiation treatment acted on differentiation steps that were specific for one of the sexes and fully developed already by the beginning of the fetal period prior to gonadal differentiation. It was speculated that lesions in the X chromosome would be involved in the development of such a specific dimorphic action, and that this pattern might only become apparent in the forebrain because of the relationship existing in this organ between cell necrosis and rosette development [S60].

119. Dimorphic lesions were described not only in the forebrain, but also in the epithalamus, as gross malformations appearing in the mouse at 18 days p.c., following a three-day fractionation treatment at doses between 0.95 and 1.35 Gy/day on days 11-13 p.c. [S61]. Two specific types of lesions regarding, respectively, the dorsal diencephalic sulcus and a narrowing of the epithalamus were recognized: it was shown that the second (and more severe) type of malformation is rather more common in female fetuses. Dose dependencies for the two lesion types were also described. The sexually dimorphic reaction pattern of these thalamic malformations cannot be explained by the fact that the relevant structures are in a different developmental stage in the two sexes at the time of irradiation; nor with a disturbance of perinatal hormone imprinting mechanisms in the CNS occurring in females more than in males. The authors believe that these malformations should be viewed together with other changes described in the neo-cortex [S60] and are the result of retrograde (cortico-thalamic) trans-synaptic degeneration, secondary to the cortical lesions. Epithalamic dysfunction in the immediate neo-natal period would then be responsible for the death of animals carrying preferentially the second type of lesions, which are the female animals.

120. In order to find some justification for such a preferential effect on female animals, Weber and Schmahl [W19] studied also the developmental profiles in mouse brain of the activities of a number of enzymes (alpha-galactosidase, hypoxanthine-guanine phosphoribosyltransferase and glucose-6-phosphate dehydrogenase) which are linked with the X-chromosome. The inactivation of this chromosome, which takes place at an early stage of development, leads to identical enzyme activities in male and female animals between 15 days p.c. and 64 days of post-natal age. These enzyme activities were studied in the brains of animals that had received 1.05 Gy three times, at 11, 12 and 13 days p.c. Treatment of the animals with this dosage (but not after 3 × 0.95 Gy or 3 × 1.15 Gy at the same ages) resulted in death of predominantly female offspring within 48 hours of birth [S59]. When the hypothesis was tested that such preferential mortality might be attributable to a reactivation of the inactive female X-chromosome in brain cells, it was found that, although the radiation-induced differences in enzyme patterns were quite remarkable, none of them was specific to either of the two sexes. It appeared unlikely, therefore, that the two phenomena might be at all correlated.

121. Schmahl et al. irradiated pregnant NMRI mice on gestational days 11-13 with 3 × 1.05 Gy and found that only the female offspring had an increased post-natal mortality within 2 days of birth [S59]. It should be noted that this effect was not observed with 3 × 1.18 Gy. However, within this narrow dose range, the only neuropathological difference between the treated groups was the occurrence of rosette-like lesions after 3 × 1.05 Gy, which were more abundant in females than in males. This was the reason to associate the post-natal mortality effect with rosette-like lesions in the brain [S60]. Weber and Schmahl [W20] investigated the weight of the brain, its protein content and the activities of two enzymes, acetylcholinesterase and Na,K-ATPase, at various post-natal ages up to 64 days. The treatment resulted in a 50% reduction of brain weight, as compared with controls, with accompanying alterations of the protein content. The activity of acetylcholinesterase was increased in treated offspring at all times, but the differences from control levels were not very high. The activity of Na,K-ATPase was changed slightly, but not significantly. Brain weight, protein content and enzyme levels were not different between male and female animals, either control or irradiated. Thus, these findings do not help in explaining the sex-specific difference.

122. Konermann [K11] exposed mice during organogenesis (6-13 days p.c.) or fetogenesis (14-18 days p.c.) to gamma rays (10-80 R/day) and studied the growth-retarding effect of the treatment on the brain. He found that under these conditions the capacity for compensating the brain growth deficit became smaller the later in development that irradiation took place. Even in the absence of appreciable embryo or fetus killing, the growth-retarding effect of the exposure continued at long term. Daily fractionated treatments at 6-10 days or 5-9 days p.c. resulted in death as well as in some deficit of brain weight. The uptake of

tritiated thymidine into brain DNA at birth was decreased in irradiated as compared with non-irradiated animals, but at the end of the first and at the end of the second weeks in neo-natal life it appeared to be increased. At this latter time the amount of uptake followed an increasing function of dose and, therefore, of the brain growth retardation. This abnormal response was interpreted as an overshoot in the recovery processes, due to the fact that microneuron and glial cell proliferation, which occurs during early post-natal development, is stimulated in inverse proportion to the radiation-induced killing of neurons.

123. Other papers by Konermann et al. [K12, K36] summarized a variety of post-natal effects induced by pre-natal and perinatal x irradiation of the albino mouse brain with doses of 0.25-0.5 Gy. Biphasic patterns of increased brain cell proliferation occurred during the time of myelination gliosis after acute or daily fractionated exposure to radiation. Such over-proliferation was, however, insufficient to make up for the loss of brain weight and was only confined to glial cells, so that the organ could not recover its specific functions. Other damages in the lipid uptake, myelination and formation of the tigroid substance were also reported. Biochemical and histochemical responses fluctuated with time, while structural and cytoarchitectural damage persisted in post-natal brain. The changes described were induced by irradiation throughout the advanced stages of intra-uterine and the post-natal development in the mouse, as might be expected from the normal pattern of development of the CNS in this species. Disruption of cerebellar architecture in mice given various doses of gamma rays from ^{137}Cs at birth (0.5 to 7 Gy) or on day 17 p.c. (2-4 Gy) was also described by Sasaki [S79]. The sensitivity of fetal mice appeared to be lower than that of mice irradiated post-partum. The changes described were of different severity and persisted at 900 days of age.

124. As to functional effects, Minamisawa and Sasaki [M42] studied the electrocorticogram (ECG) of hybrid mice irradiated at 17 days p.c. or at birth with x rays (300 R). The ECGs were recorded on the freely moving animals by permanently implanted electrodes fixed over the surface of the visual cortex when the animals were 6-8 or 24-26 months of age. From the pattern of changes observed, the authors concluded that the adult and aged mice, irradiated when in utero or at birth, showed ECG responses that were characteristic of later ages in control groups. This was interpreted as evidence of an accelerated "aging" of the ECG pattern brought about by the radiation treatment. In its 1982 report [U5], UNSCEAR commented at length on the impossibility of defining "aging" at the tissue or organ level by means of any morphological or functional test.

(b) The rat and the guinea-pig

125. In an attempt to reconcile differences between morphological and teratological findings after x irradiation, Berry and Rogers [B15] provided a description of the development of the rat cerebral cortex, studied by labelling with tritiated thymidine between the 16th day in utero and birth. They were able to establish that the neuroblasts present on days 16-17 migrated to infragranular layers V and VI; those found on day 18 to layer IV; and those found on days 19-21 to layers II and III. The ependymal cells retained a physical connection with the superficial and deep strata of the cortex by means of their long processes, which provided a channel through the cortex along which the neuroblasts migrated. Not until completion of migration did these cells separate from the ependymal processes to start differentiation. Therefore, in this species also the formation of brain cortex occurs from the inside out, as was later shown in primates and man (see I.B.2).

126. In contrast with the paucity of data on the development of the cerebrum in the rat [B15, B30, B38], there is relatively more data available on cerebellar development [A12-A14, L10]. It may be deduced from the whole of the information cited that the growth spurts of the cerebrum and cerebellum are very similar in the rat and the mouse; and that while the cerebrum shows a sharp peak of mitotic activity around 14-15 days p.c., the cerebellum shows its most active wave of cell production 6-10 days after birth. Any study that does not take into account the peculiarities of each structure is therefore bound to be inaccurate, considering the developmental complexity of the phenomena described.

127. Berry and Eayrs [B30] described the effect of x irradiation of the fetus (200 R) on the histogenesis of the cerebral cortex and the morphology of neurons. Exposure at 19 days of gestation resulted in widespread destruction of cortical cells 12 hours after irradiation, with signs of recovery and differentiation of the surviving neurons at 24 hours. Evidence was also found that under these conditions the ependymal layer had lost its capacity to contribute further, as in the normal situation, to the histogenesis of the cortex. A variety of morphological cell abnormalities were described in the irradiated neurons. The germinal layer, the mantle layer and the actively migrating cells appeared to be selectively destroyed, while cells which had completed migration remained relatively unharmed. The distribution pattern of dendrites appeared to be little affected by irradiation, although the number of primary dendrites and the amount of branching was less than normal, suggesting that the capacity of these cells to establish interconnections with incoming axons was greatly reduced.

128. According to Semagin [S9], exposure of pregnant Wistar rats from day 16 of pregnancy to delivery into a ^{60}Co irradiation field between 0.0008 and 10 R/day (8 hours/day) reduced the whole-body weight of the new-born animals and increased significantly the absolute weight of the brain. The effect could not be attributable to oedema. Thickening of the cerebral cortex (in the absence of any microscopically detected destructive changes) was also described. The author attributed this finding to a stimulating effect of low-dose-rate irradiation on the cerebral cortex and was obviously aware of the peculiarity of this conclusion in the face of other experimental and clinical data. The same author [S75] irradiated 26 non-inbred rats

in utero on the 18th day of pregnancy (1 R) and tested them (together with 25 controls) for conditioned reflexes, starting at 45 days of age. In the irradiated animals, there was a slowing down in the consolidation of conditioned reflexes and a weakening of such reflexes. Conditioned natural reflexes were found to be more pronounced than in the controls, a phenomenon explained by an impairment in the co-ordination of movements. Irradiated animals also showed an increase in the magnitude of intersignal reflexes during conditioning of differentiated responses. In the author's opinion all these findings are evidence for the harmful effect of the radiation treatment.

129. Inouye [I1] studied, in the cerebral cortex of rat embryos exposed to 200 R at 17 days p.c., the development of histopathological changes from 1 hour p.c. to late in post-natal life. Cell killing of the brain cortex was described as an early change which later resulted in profound disturbances of the fibre formation of the corpus callosum, with reduction of its size. These defects were also recognizable in irradiated animals which reached adulthood. As to the cortex, disturbances in cell migration were seen, resulting later in a hypoplasia of layers II-IV, abnormal branching and irregularity of the dentritic processes of pyramidal cells in layer V. Tridimensional reconstructions of various sections of the brain showed marked hypoplasia of the cortex, the basal ganglion and the thalamus.

130. Following a suggestion that there may be species differences in the relative contribution of the different layers of the neocortex to the development of the corpus callosum, Jensen and Altman [J5] made use of the sterilizing effect of x irradiation given at various pre-natal ages to kill the late-generated neurons in the rat. They found that the late-generated neurons residing primarily in the supra-granular layers are essential for the formation of the corpus callosum. Reyners et al. [R29] irradiated with x rays on the 15th day p.c. rat embryos with doses of 0.1-0.5 Gy, and examined them at various times between 1 month and 2 years after irradiation. They found that the depth of the cingulum bundle, a myelinated substructure of the corpus callosum, was a sensitive indicator of radiation damage at doses of 0.2 Gy. The authors traced the origin of the anatomical damage to a disorder in the sequence of morphogenetic events, rather than to a process of radiation-induced cell killing.

131. In experiments involving the use of ^{137}Cs gamma rays (1.75 ± 0.08 Gy) administered to pregnant Sprague-Dawley rats on gestation days 16-19 (day 1 was considered to be the morning after mating), Jensen and Killackey [J13] investigated the subcortical projection of cortical neurons that had been caused to take up an ectopic position by the radiation treatment. The study was carried out by a labelling technique with horse radish peroxidase applied when the rats had reached 30-80 days of age. It showed that the radiation treatment given before, during, or after the production of neurons destined for layer V in the neocortex and projecting to the spinal cord resulted in their abnormal location. One ectopic location, typical of rats irradiated on or before day 17, was in clusters beneath the cortical white matter bordering the dorsomedial aspect of the lateral ventricle. The other location was between layer V and the pial brain surface, which was seen in rats exposed at any time between day 16 and 19. In spite of their abnormal position, all these neurons projected to a sub-cortical target appropriate for level V, indicating that neither the migratory path nor the final position of the neurons was essential for specifying their target. Furthermore, the fact that peri-ventricular ectopias occurred after irradiation on day 17 and earlier suggested that the projections of the cortico-spinal neurons are determined early in their individual ontogeny, prior to migration. In view of the high dose used, the importance of this paper lies more with the implications for mechanisms, rather than with any consideration of risk.

132. Adult rats exposed in utero to x rays (0.09 to 0.49 Gy) on day 15 p.c. were killed at various ages for electron microscopic analysis of different brain parameters selected to provide quantitative information. The x-ray treatment reduced the body size of both nerve and glial cells in the central cortex and produced an increased packing density of these cells. The reduction in cell size appeared to be the primary factor in these changes because, when the data were appropriately corrected, the number of cells per unit volume of cortex was not significantly modified by the radiation treatment. It appeared as though the brain had suffered a sort of miniaturization process, rather than a loss of cells as a result of cell killing. Defects of synaptic arborization were also described. A dose-related decrease of blood capillaries in the cortex was reported from these studies, which, when appropriately corrected for the reduction in cortex thickness, became statistically significant at the dose of 0.49 Gy [R25].

133. A comparison of the neuronal damage induced by irradiation in chick and rat embryos was attempted by Schneider and Norton [S8]. They carried out an analysis of cells at the telencephalic-diencephalic border on neurons which show morphologic similarities in the two species. Analysis involved nuclear size, the content of cytoplasmic acetylcholinesterase and the presence of several branched, spiny dendritic processes. These authors were able to establish that 1 Gy in the 7-day chick embryo had about the same effect as 1.25 Gy in the 15-day rat fetus. They interpreted the similarity of the response of an avian and a mammalian species to mean that maternal factors in mammals may be of little significance for the radiation response of these structures.

134. A fair amount of information has also been reported in regard to cerebellar damage. Das [D3] exposed 18 days p.c. rat embryos to x rays (170 R) and collected specimens of the cerebellum at various times thereafter, from 1 hour to 30 days post-natally. Six hours after irradiation, the author described pyknosis of cells in the external granular layer along the posterior part of the cerebellum. The neuroblasts, that would later develop into Purkinje cells, were found arrested in their migratory pathway. Some recovery of the external granular layer was seen at later stages, but

an abnormal clustering of neuroblasts resulted as a permanent architectural abnormality during post-natal development. Malformation of the folia in the cerebellum, and the smaller size of this organ, were described as the long-term sequelae of the treatment.

135. Given that development of some brain structures continues in the rat well after birth [D19] it is not surprising that similar changes could also be induced by post-natal irradiation of the rat 5 days after birth. According to Korogodin and Korogodina [K14], animals exposed to various doses of ^{60}Co gamma rays, and killed at 30 days of age, showed disorganization in the location of the Purkinje cells which was dose-related with a maximum at the low lethal doses. Here again, the increase of disorganization was accompanied by a decrease of the cerebellar weight and an impairment of some electrophysiological parameters.

136. In experiments by Inouye [I6], a quantitative analysis was attempted of the cerebellar malformation induced by exposure to x rays (100 or 200 R) administered to pre-natal rats on one gestation day from 16 to 21 p.c. The changes described were those to be seen at 60 days of post-natal age in surviving animals. After the highest exposure, there was a slight reduction of the weight, but not of the width, of the organ. The dorso-ventral length of the cerebellum was reduced, the more so the earlier x irradiation had taken place. A description of the diameters and arrangements of hemispheres and lobules, as altered by the radiation treatment, was provided. Histologically, an ectopic appearance of Purkinje cells in either the granular cell layer or the white matter was seen in rats treated on days 20 or 21 p.c., but not for irradiation at earlier stages. The size and the architectural disposition of the cerebellum after 100 R were only mildly altered.

137. Inouye and Kameyama [I7] studied the effects on the matrix cells in the cerebellum of rats exposed to several single doses of x rays ranging from 0.03 to 1 Gy. Exposure took place at 0, 3, 6, 10, 13 and 17 days of post-natal age. Animals were killed 6 hours after exposure and the organ was processed for scoring of the frequency of pyknotic cells in the external granular layer along the primary fissure. Pyknosis was significantly increased by the lowest dose level of 0.03 Gy. At all irradiation times, the incidence of pyknotic cells increased linearly with dose in the range of 0.03 to 0.25 Gy and then increased more rapidly at higher doses, suggesting a linear-quadratic trend. The highest response was seen in animals irradiated at 6 and 10 days of age.

138. A quantitative analysis of cerebellar development was performed by Inouye and Kameyama [I10] on F334/DuCrj male rats exposed between 0 and 17 days post-partum to single whole-body doses of 0.25, 0.5 and 1 Gy of x rays. When these animals reached the age of 8 weeks, they were killed and measured for body, brain and cerebellum weight. None of these parameters, nor their ratios, appeared to have been altered by the treatment. Microscopically, the only effect reported was a reduction of cell counts in the molecular layer of the cerebellar decline. This was statistically significant only in animals given 1 Gy at 6 days of development and amounted to about 17%. There was no gross cortical abnormality to be observed in the cerebellum.

139. Concerning behavioural functional effects, experiments were reported in which rats were exposed at 17 days of gestation to x irradiation (150 R, 200-kVp) and males were reared after birth under enriched (EC), standard (SC) or impoverished environmental conditions (IC) for 30 days after weaning [S57]. Standard conditions implied large cages with three or four rats; impoverished conditions implied isolated cages with animals housed singly; and enriched conditions implied cages where many animals lived together and had various objects to play with, such as ladders, tubes, balls and boxes. The irradiated animals, together with an appropriate number of controls, were then tested in a maze for their performance, using a standardized behavioural technique. The effects of radiation and of the different environmental conditions were both significant in initial, repetitive and total error scores and running time. At later times, it was found that the differences between the EC-SC and EC-IC were significant in irradiated rats, whereas only the EC-IC difference was significant in controls. A 20% decrease in brain weight was present in irradiated animals, as compared with controls. X-irradiated animals, compared with controls, revealed a decrease in total protein, protein per gram of cortex, total benzodiazepine and muscarine receptor bindings and muscarine cholinergic receptor binding per milligram of protein in the cortex. However, all these indices were not significantly different in animals reared under different environmental conditions. Other experiments by the same group had shown that at 200 R the radiation effects were very evident and those of the environment virtually absent [K35], while at 100 R irradiation was essentially insignificant and environment very important in respect of the performance of irradiated microcephalic animals [S73]. The conclusion from the experiments on the intermediate 150 R exposure was that environmental enrichment is a useful tool to alleviate the learning impairments induced by irradiation in pre-natal animals.

140. In other experiments by Tamaki and Inouye [T8], a facilitation of the avoidance behaviour was found in rats that had been exposed to x rays (200 R) on their 17th day of gestation. The primordial hippocampus of these animals showed extensive cellular damage. The facilitation described was thought to be a consequence of a strong tendency to running induced by the shock and was related to the hippocampal damage.

141. Bornhausen et al. [B17] studied the learning ability of rats and reported data on animals irradiated in utero on days 6-9 of pregnancy with 4 daily doses of 0.01 Gy. When tested at 4 months of age, using behavioural tests in which the performance requirements were gradually increased, the animals showed a significantly impaired function, particularly when they were required to perform the most difficult tests.

Preliminary data were also reported by the same author [B40] on rats irradiated in utero on day 13 of pregnancy with doses of gamma rays from ^{137}Cs in the region of 0.15-0.9 Gy. In addition to showing a large variability of performance from one animal to another, the data indicated that performance deficits depended more on the time of exposure than on dose.

142. In another paper [M17], the locomotor damage present in adult rats after irradiation in utero (days 14-17 p.c.) was studied. Alterations in gait were found after 125 R given on days 14-16. Exposures of 50 R on day 14 and 125 R on day 17 induced no effect on locomotion. Hopping or alternating, waddling gait, accompanied by abnormal positions of the hind feet, resulted from such treatments. By histological examination of the brain, these effects could be traced back to lesions of the corpus callosum and the telencephalic commissures.

143. Hudson et al. [H5] reported that neo-natal x irradiation induces, at 7 days of age, changes in the metabolism of monoamines in the brain of rats. The concentrations of norepinephrine and serotonin increased in all the regions of the CNS showing rapid growth and proliferation of the axons, but not in the regions of the cell bodies from which these transmitters originate. The increase of the concentration was attributed to the increase in the rate of synthesis. These changes were to be seen up to about 3 weeks of age, but the monoaminergic system returned to normal when brain maturity was attained. Such changes were interpreted as reflecting a disturbance in the density of nerve endings in the regions where these terminate due to the radiation-induced disturbance in cell proliferation. Cerda et al. [C10] irradiated young rats at 5, 10 or 25 days of post-natal life with doses of 1-8 Gy of ^{60}Co gamma radiation and followed the fate of the DNA strand breaks in the brain cells between 1 and 180 minutes of irradiation. The number of breaks induced per gray was found to agree with earlier data on small intestine and spleen mouse cells. It was also found that by 30 minutes after treatment the radiation-induced double-strand breaks in the brain were repaired in all age groups tested.

144. Wanner and Edwards [W12] reported on a comparison of the effects of ^{60}C gamma radiation and hyperthermia in inducing retardation of brain growth in the guinea-pig. To this end, they exposed pregnant animals on the 21st day of pregnancy to gamma-ray doses of 0.32-3.75 Gy delivered at dose rates of 0.043, 0.069 or 0.125 Gy/minute; or to 42.5-43.5° C for one hour. The offspring were killed within 24 hours of birth for examination of the viscera and weighting of the body and brain. Doses of 0.04-0.99 Gy produced a dose-related, irreversible reduction of the brain weight, but had little effect on the weight of the body. Hyperthermia also resulted in microcephaly of the offspring. The relative effectiveness of the two agents was such that a dose increment of 0.525 Gy produced a brain weight loss equivalent to an elevation of the maternal temperature of 1° C. The dose threshold detected by this assay system was between 0.05 and 0.10 Gy of gamma radiation.

(c) Primates

145. In view of the difficulties in obtaining samples from irradiated human fetuses, observations on primates are of particular interest. Brizzee et al. [B8] reported on the gross and microscopic effects in some brain structures (visual cortex, hippocampus and others) of squirrel monkeys irradiated at each trimester of their pre-natal life with ^{60}Co gamma radiation (2 Gy). The total duration of pregnancy in these animals is 165 ± 5 days. In the visual cortex, animals irradiated during the last two trimesters and examined 2 days after birth showed a thinning and a decrease in the number of neurons per cubic millimetre of tissue, although neither of these effects was statistically significant. The spines in the dendrites of the giant Meynert neurons also showed some alterations. In the hippocampus, the thinning of the cortex and the loss of neurons in the irradiated monkeys was statistically significant, as were the alterations in the morphology of dendrites. In the three animals irradiated in the first trimester, gross abnormalities of the brain, urinary system and limbs were seen, but no microscopic observations of the brain were yet available. It is to be hoped that, with an improvement of the morphometric techniques, the careful quantitative evaluation of similar data on primates will facilitate the projection to man of findings from the widely studied rodent species.

146. In another paper by Brizzee and collaborators on the effects of pre-natal irradiation of the squirrel monkey [B10], various morphological and functional end-points were evaluated. Data were reported on 78 pregnant females, either controls or irradiated with ^{60}Co gamma rays (0.1, 1.0 and 2.0 Gy). Irradiation took place at 95 ± 5 days of gestation. Among all females, 55 live births and 23 infant deaths were recorded. Up to the time of reporting, the sex ratio and the percentage of infant mortality were not significantly different between controls and irradiated. On day 2, and during 30 days after birth, body weight, crown-rump length and head circumference were decreased in the offspring receiving 1 and 2 Gy. Various nervous reflexes were also disturbed, as were other tests of behavioural exploration and general motor activity. Temperature was significantly lower, and the respiration rate significantly higher, in these animals. Brain, cerebellum, pituitary and kidney weights were significantly lower after 2 Gy on day 2 after birth. Dendritic spine counts of cortical motor neurons were also significantly reduced after the same dose. The percentage of B lymphocytes with surface IgM and the total blood lymphocytes were decreased after 1.0 Gy at 1 year of age.

147. The same group [O2] reported on the effects of pre-natal ^{60}Co irradiation on the visual acuity, maturation of the retinal fovea and the striate cortex of squirrel monkey offspring receiving between 0.1 and 1.0 Gy at 80-90 days of gestation. The visual acuity of animals receiving 0.5 and 1.0 Gy was significantly lower than that of control animals or of those given 0.1 Gy: these changes were scored in the periods from birth to 30 days and from 30 to 90 days. Morphometric evaluations of the retina at 90 days of

age showed that pre-natal exposure, with a dose of 1.0 Gy, resulted in a significant difference in foveal cone density. In the brain there was a corresponding thinning of the foveal striate cortex at 90 days after 1.0 Gy. The cytoarchitecture of the cortex was also altered.

148. Finally, in another paper [O1], the effects of 0.5 and 1.0 Gy of gamma rays from ^{60}Co given to squirrel monkeys pre-natally between 80 and 90 days p.c. were reported. The animals were studied between birth and 3 months of age for a number of morphological and functional parameters. The circumference of the head, the crown-rump length and (to a lesser extent) the whole-body weight grew at a significantly slower rate after irradiation. The nervous activity of the monkey irradiated at both doses appeared to be less accurate and complete by comparison with that of the controls; the time required for the performance of reflexes and neuromuscular co-ordination was also lengthened. In visual orientation, discrimination and reversal learning, irradiated animals had a significantly decreased percentage of correct responses. Spontaneous light-dark activity was significantly increased in the dark session over that of control. Plasma cortisol was also significantly increased in the animals receiving 1 Gy.

4. Other types of malformations

149. A previously undescribed class of malformations of the skull was reported by Schmahl et al. [S58] on NMRI mice exposed in utero in the late organogenesis stages (11-13 days p.c.) to either a single (200 R) or fractionated (3 × 160 R) x-ray exposure. The developmental stage at irradiation appears to be a critical factor for observation of these effects, because irradiation during the early fetal period (14-16 days p.c.) did not influence the development of the neurocranium, irrespective of the dose applied. The malformations consisted of: (a) small nodules of cranial hyperostosis in up to 90% of the offspring; or (b) an excessive formation of bony tissues, a heterotopic chondrification of the neurocranial capsule in the parietal and occipital bones. Both classes of abnormalities occurred in the presence of a completely normal development of other cranial and facial bones. Malformations of type (b) extended deep into the forebrain and could be seen histologically only in about 10% of the offspring. A growth disturbance of both the mesenchymal skull primordium and the brain was thought to be at the origin of these overgrowth phenomena, in the sense that brain malformations producing a lower intracranial pressure may lead in turn to an abnormal growth activity of the skull sutures, stimulated by irradiation in a hyperplastic direction. For these effects, see also a paper by Meyer [M44].

150. Fractionated irradiation of pre-natal mice in late organogenesis at 11-13 days p.c. induced a marked incidence of hypotrichosis and alopecia in the offspring and led to severe ulcerative dermatitis, starting at 2 months of age. This effect had a marked prevalence for animals irradiated at least on the above days (other fractionation patterns were also tested extending over 11-16 and 14-16 days p.c.). There was no apparent dose dependence for such an effect within the range of 2.4 to 7.2 Gy total dose, as incidences of ulcers of between about 39 and 48% were observed among the irradiated animals. No sex dependence was apparent, nor was any dependence on the conditions of the animals' housing. Internal organs showed no special sign of alterations, except for a higher incidence of amyloidosis. Keeping animals under germ-free conditions also did not alter the disease pattern or incidence [S62]. The intra-cutaneous administration of skin extracts from affected animals into non-irradiated ones resulted in a marked infiltration of leukocytes, suggesting that the pathogenesis of the lesions may involve a radiation-induced skin dysplasia which is followed by an endogenous leukotactic activity resembling human psoriasis [S63].

151. An observation by Michel and Fritz-Niggli [M8] should be mentioned for its general methodological relevance. These authors studied the effect that confinement and restraining of pregnant mothers (which is often necessary in the course of experimental work) may have on the incidence of malformations in the developing fetus. NMRI pregnant females on day 8 were restrained for 2 to 36 minutes: the fetuses they carried on day 13 had a significantly higher incidence of malformations of all types (but not of any single class). The effect was attributed by the authors to a hormonal discharge of the hypophysis-adrenocortical axis induced by the stress of confinement.

B. DATA FROM MAN

152. Müller and O'Rahilly [M57] published a histological study of a twin at stage 13 showing an opening of the neural tube, believed to be the earliest example of purely cerebral dysraphia recorded so far. The paper is valuable for its careful description of timing of morphological events and mechanisms resulting in anencephaly in man and for a comparison, by means of synoptic tables, of events and stages leading to similar types of malformations in the rat and mouse.

153. The information accumulated since 1977 on the occurrence in man of severe mental retardation following exposure at Hiroshima and Nagasaki is of great interest and must be reviewed in depth. In 1978, Ishikawa [I2] published what is essentially a case report on the long-term follow-up of four patients exposed pre-natally to the atomic explosions and followed for their psychological symptoms over a long time. These individuals had been exposed between 2 and 8 weeks of gestation at distances between 780 and 1180 m from the hypocentre and their mothers had all shown signs of acute radiation sickness. The subjects were between 1.3 and 2.0 kg of weight at birth and were later found to be microcephalic, and physiologically and mentally retarded. Degenerative signs and diseases, as described in the "Epidemiological study on microcephaly" [T5] were also found in these children. This small survey showed essentially that the state of these children was clinically serious and their prognosis over a long observation period of about 20 years should be considered as very severe.

154. Ishimaru et al. [I12] published a re-analysis of the anthropological measurements obtained at age 18 in children exposed in utero (and their controls) in Hiroshima and Nagasaki. The paper reported, as a function of dose, the height, body weight and head and chest circumference of these children. There was a statistically significant linear decrease of head and body size with T65 dose for both sexes and both cities (except for height in Nagasaki females), but the inter-relationships between the various effects shown was not analysed. There was no apparent correlation between pre-natal age at exposure and head circumference. It should be noted, however, that head circumference was not adjusted for body size, and pre-natal age was treated as a continuous variable, without regard to discrete embryological events.

155. A discussion of the terms "microcephaly" and "mental retardation" is to be found in a paper by Dobbing [D21] who pointed out that such terms are used for conditions of different clinical severity in various reports. Radiation is very effective in producing small head size and mental handicap, but it is only one among many other agents that may cause similar effects. Actually, the precise developmental time at which an agent responsible for such effects is applied is often more important than the nature itself of the agent. Contrary to other teratological malformations, which are induced by a precise, momentary exposure to such agents, microcephaly and mental handicaps are characterized by relatively longer periods of developmental vulnerability lasting hours or days in rodents and other fast-growing species, and weeks or months in man and slowly growing animals. Unlike teratological defects which result in permanent lesions, brain growth disturbances may to some extent be remedied, and much more so the later the affected stage is and the earlier any remedial action is undertaken.

156. A study by Otake and Schull [O3] describes epidemiological findings in atomic bomb survivors in relation to dose and time, thus allowing some rough estimate of the rate of incidence of mental retardation. After exclusion of a few children from the in utero sample at the Radiation Effects Research Foundation (RERF), on account of wrong or incomplete information on date of birth and exposure of the mothers, the sample on which the study was based comprised 1599 children (1251 at Hiroshima and 348 at Nagasaki). For the purpose of the study, mental retardation was defined as incapability "to perform simple calculations, to make simple conversation, to take care of himself or herself, or if he or she was completely unmanageable or had been institutionalized". Most of the retarded children were never enrolled in public schools, but among the few who were, the highest IQ was 68.

157. The stages of pregnancy at the time of the bombing were re-assessed and expressed as the number of weeks elapsed from the presumed time of conception. The dose to the fetuses was expressed in terms of maternal kerma T65 rad, modified by the related attenuation factors for position of the fetus within the body of the mother (both at present under revision), because this was likely to remain, for some time, the only system of individual dose assessment. The alternative provisional "free-in-air" doses available in 1983 did not appear to change greatly the form of the dose relationships or the estimates of incidence. A detailed account of the findings is given in Table 9.

158. Table 9 and Figure XI show a dose-related increased risk of mental retardation for gestational ages 8-15 weeks and also, to a lesser degree, for ages 16-25 weeks. Table 10 shows the statistical parameters for the evaluation of the relationships between mental retardation and fetal absorbed dose from reference [I11]. It shows that the absolute excess risk per unit absorbed dose for exposure at 8-15 weeks in both cities combined, when zero-dose cases are excluded, is $0.38 \pm 0.09\ Gy^{-1}$; this value is about 4 times greater than for exposures at 16 weeks and over. Statistical analysis showed that there was little or no suggestion of a non-linear component in the dose-response function at 8-15 weeks. The calculated regression suggests no threshold. Actually, a threshold of > 40 mSv may be excluded only with a probability of 0.20 [P17]. This is not true, in general, for the later exposure times, particularly after 25 weeks of conception, but here the dose-response relationship is weak and not significantly different from zero. Exclusion of 4 mentally retarded cases non-radiation-related (3 with Down's syndrome and 1 with infantile encephalitis) did not alter the above findings appreciably. Although children exposed in utero in the range between 2000 and 3000 m from the hypocentre were not included in this revision, ancillary evidence showed that there was no reason to suggest that the frequency of mental retardation in this group (approximately 5/601 or 0.8%) would be different from that found in the control group (9/1085 or 0.8%). As to the difference between observations in the two cities, the baseline values of 0.6% incidence of mental retardation at Hiroshima and 1.6% at Nagasaki are not significantly different but, if so, could not be explained on any known ground. Differences in other irradiation groups also remain unexplained. Moreover, if the absolute risk of mental retardation per unit dose seen in the more numerous Hiroshima series were used to predict the incidence among those exposed at Nagasaki, given the average dose received by that group, 4 to 9 cases (all ages included) would be expected to appear in that series; 4 cases were actually found.

159. Concerning any possible correlation between mental retardation and small head size, it has been reported [W22, W23] that about 10% of individuals having a head size two or more standard deviations below the mean of all those in the study sample were also mentally retarded. Data on the relationship between mental retardation and small head size are given in [O4]. This subject has been discussed separately by Mole [M9, M10, M21]. It appears that among the mentally retarded children, about 60% had a small head size. Thus, the association between the two conditions is not very close, owing presumably to the fact that loss of neuronal cells is compensated to some extent by over-proliferation of the glia, which may restore the size, but not the function, of the irradiated brain. There is no unequivocal evidence that other types of malformations may be increased in this population of children exposed in utero [M51, P16].

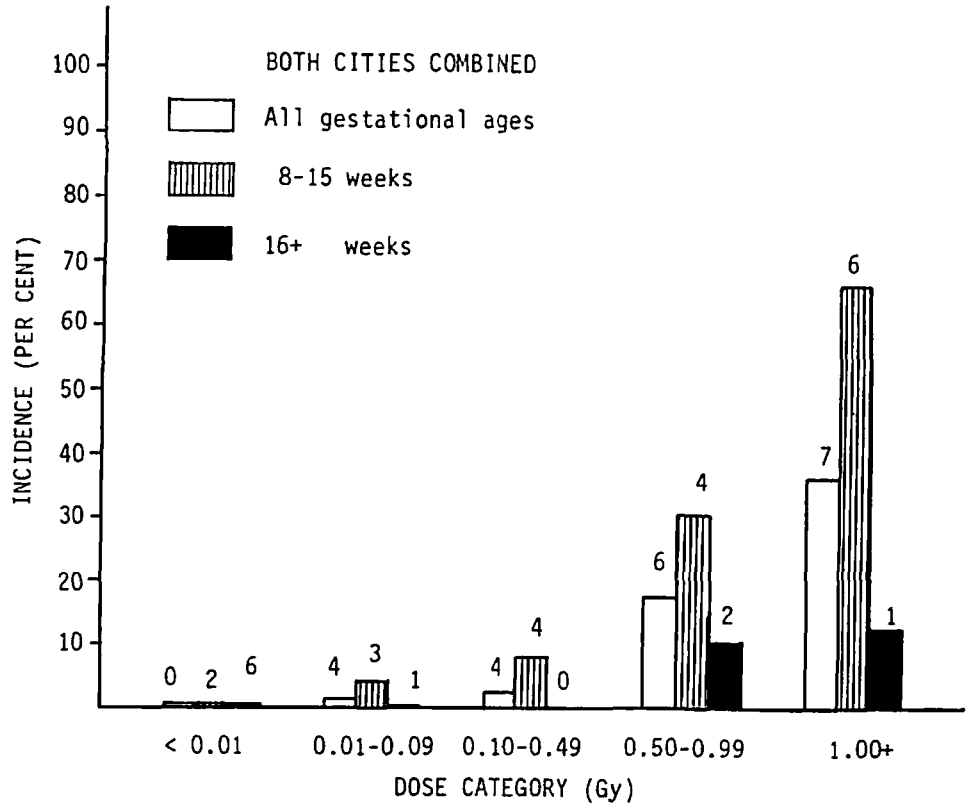

Figure XI. The frequency of mental retardation among children exposed in utero during the atomic explosions in Japan, as a function of dose category and gestational age. (Both cities combined.) [111]

160. The existence in this series of a well-defined period of maximum susceptibility of the brain, taken in conjunction with the changing form of the dose-effect relationship as a function of the time at exposure, were discussed in the study by Otake and Schull [O3] in light of the findings reviewed in chapter I, section A. The evidence presented there shows that both proliferation and migration of neurons in the human forebrain are largely completed by the 16th week of gestation, although glial cell production may still continue in the brain cortex up to much later stages in development, with some uncertainty as to the timing and the actual extent of such processes. Also, any distinction between effects on cell proliferation, differentiation and migration is hard to make since these are not taking place at the same time but different cells in different stages are found in the cortex at the same time of development.

161. The apparent linearity of the response at 8-15 weeks warrants some discussion. In view of the non-stochasticity of the phenomena, which is reflected almost invariably in the animal experiments by the curvilinearity (upper concavity) of the dose-response relationships for malformations induced at well-defined developmental stages (see III.A), the finding of a linear trend with dose appears surprising. There are, however, possible explanations for it. Among them: the fact that many cellular events (division, differentiation, migration) may be variously contributing to the observed effect: the fact that the period between 8 and 15 weeks of gestation is very long and that the sensitivity of the neuroblasts and their functions may change considerably during this time; the fact that the clinical conditions grouped under the definition of "mental retardation" may be of different severity, and that severity may change with dose; the fact that, in clinical judgement, severe mental retardation is an arbitrary cut-off point in the lower portion of a normal distribution curve; and the possible effect of the environmental conditions under which retarded individuals were kept from birth to diagnosis, which might themselves have affected the outcome of any given case. If any of the processes or conditions envisaged were characterized by a curvilinear relationship with dose, but if each of these relationships had different linear or quadratic components, it would still be possible for them to combine in a way that the overall effect might be seen as apparently linear. However, in the present state of knowledge, the linearity of dose-effect relationships from 8 to 15 weeks of gestation must be taken as observational evidence, since our capacity to interpret it in precise radiobiological terms may be inadequate given the complexity of the underlying phenomena.

162. The data reported by Otake and Schull [O3] show quite conclusively that the apparent absolute excess incidence of severe mental retardation per unit dose, under the exposure and observational conditions described, is of the order of $4\ 10^{-1}\ Gy^{-1}$ at the peak sensitivity period, a figure greater than that given by UNSCEAR in its 1977 report [U2] which was averaged over the whole pregnancy and based on an earlier and much less refined analysis of the epidemiological evidence. They also show that the develop-

mental stages at 16 weeks or later, which coincide in man with the period of synaptogenesis, are distinctly less sensitive to the radiation insult (risk factor about 10^{-1} Gy^{-1}) and that the form of the dose-effect relationship for mental retardation induced at these stages does not exclude curvilinearity. This latter observation would be expected with a non-stochastic type of damage; the decreasing susceptibility with time is to be seen in the framework of the repeatedly discussed concept of maximum sensitivity periods and the time for the changing form of the response agrees closely with the nature and timing of developmental events in the brain cortex (see Figure V).

163. The results of an autopsy performed on a microcephalic child exposed in utero to the atomic bomb deserve some attention, even though they are so far only available in an abstract [N12]. As previously discussed, disturbance of neuronal migration may be an important cause of radiation-induced mental retardation. The morphological consequence of disturbed migration is, of course, the presence of heterotopic gray matter in the brain white matter. The importance of this case (the dose was estimated at 1.75 Gy) is due to the finding of massive inclusions of heterotopic gray matter in the brain hemispheres. This observation confirms in man observations in animals discussed in the 1977 UNSCEAR report [U5], showing the similarity of the basic mechanisms leading to brain abnormalities and the consequent functional defects in all mammals.

164. Another aspect of these findings which deserves mentioning is the relatively high frequency of mental retardation in the control children (of the order of 1%) over lower values reported from other sources. There is, of course a well-known geographic and ethnic variability in the natural incidence of malformations (see I.C.2), to which the high rate of first-cousin marriages in Japan at the time of the bombing may have contributed to some extent. Aside from this, however, the possible effect of malnutrition has been discussed, which was known to be prevailing in Japan towards the end of the Second World War [O4]. There are data on animals indicating that malnutrition may itself produce abnormal development of the brain and brain functions [D18, W13, W32], but no clear effect of synergism with radiation has so far been documented. The extent of malnutrition in the pregnant mothers, and its effect separate from that of radiation in producing mental disorders, would be difficult to quantify and resolve.

165. Another possible confounding factor could be anaemia induced in the pregnant mothers that were exposed to a relatively high radiation dose. While indirect effects on the fetus through the mother's state of health may not be definitely ruled out, particularly at high doses [U2], there are at least some grounds for believing that they may not have played a very major role. Actually, it is known that, to a large extent, lack of haemoglobin concentration or red blood cells in the mother's circulating blood may be compensated by physiological adaptation of the haemodynamic conditions. In addition, mothers carrying congenital anaemic diseases are not known to produce an abnormally high prevalence of mentally retarded offspring. It seems likely, therefore, as a first approximation, that the high incidence of mentally handicapped children after irradiation in utero may be attributed to a direct action of radiation on the developing nervous structures of the conceptus.

166. A preliminary analysis of intelligence test scores on individuals exposed in utero at Hiroshima and Nagasaki has also been reported [S38]. In the words of the authors, the following is a summary of the major findings: "First, neither tests of skew nor graphical representation of the data suggest a commingling of distributions such as might arise through the inadvertent inclusion of a qualitative different group of individuals, that is unrecognized cases of mental retardation. Indeed, the cumulative distribution suggests a general phenomenon, a shift in the distribution of scores with exposure. Second, there is no evidence of a radiation-related effect on mental retardation or intellection generally for those individuals exposed in the first eight weeks of life. Third, the mean test scores but not the variances are consistently significantly heterogeneous among exposure categories for those individuals exposed at 8-15 weeks after conception, and less heterogeneous for those groups exposed at 16-25, or 26 or more weeks of age. Fourth, regression analyses indicate that among those in utero individuals exposed either at 8-15 or 16-25 weeks of gestational age a significant decrease in intelligence test score occurs with increasing exposure. This obtains whether the eight clinically diagnosed cases of mental retardation to which allusion has been made are or are not included. However, the shape of the dose-response curves appears different: it is linear-quadratic for the 8-15 week group and linear for the 16-25 week group. This is the opposite of the findings with respect to clinically diagnosed mental retardation in the Revised PE-86 sample. Finally, fifth, within the most sensitive group, that is, individuals exposed 8 to 15 weeks after conception, and with the better fitting model, the linear-quadratic, the diminution in intelligence score is 21-26 points per gray . . ." (at 1 Gy). Further analysis and evaluation of these data will have to be deferred until a definitive publication appears.

167. In the present state of knowledge, the action of radiation on the cortical brain function could be visualized as a dose-related shift towards lower values of the normal distribution of "intelligence" as assessed by conventional test scores. Such a shift would take an increasing percentage of individuals at the lower end of the distribution into a region where they would be clinically defined as mentally retarded. As might be expected of a true non-stochastic effect, both the probability of entering such a region and the severity of the retardation would thus be dose-dependent. However, as the clinical definition of mental retardation includes conditions of different severity, it would be difficult to predict the shape of the dose-response relationship for mental retardation. If such an interpretation is accepted, then "severe mental retardation" may be viewed as the extreme consequence of a dose-related loss of mental fuction. Accordingly, irradiation at low doses (say, 0.1 Gy of low-LET radiation) would not result in an increased probability of perfectly normal individuals becoming severely mentally retarded, but

rather in an increased probability that individuals who would otherwise develop to be at the lower tail of the distribution of intelligence test scores may suffer damage that would turn them into mentally retarded, according to the clinical definition of this term.

168. In view of the non-stochastic nature of the damage caused by irradiation in utero, one would expect conditions of lesser clinical importance than severe mental retardation to be present in the cohorts of Hiroshima and Nagasaki exposed pre-natally. In addition to the intelligence test scores mentioned above, it is known that clinical evidence collected from these children over the years is available in respect of a number of other topics, as follows: anamnestic observations, such as the occurrence of convulsions, diplopia, blurring of vision; observations on major landmarks of physical development, such as the ages when the children were able to stand up, to walk, to speak; information on school performance during elementary training (including periods of absence from school on account of disease or other causes), number and types of subjects taken at school with relevant scores over the years, performance as a function of age as the children moved to higher classes, behaviour of the children while attending school; and information on grip dynamometer studies and on repetitive action tests. All these data should give a fairly good representation of the development, neuro-muscular coordination and higher nervous function in the children [S78]. It is to be hoped that this material will be appropriately collated and analysed, because it may give information on man that is of the highest interest and obtainable directly.

169. Granroth [G8] reported a study based on the Finnish Register of Congenital Malformations, which has been in operation since 1963. Defects of the CNS (710 cases from 1965 to 1973 comprising: anencephaly, 199; spina bifida, 239; hydrocephaly, 216; microcephaly, 41; hydroanencephaly, 15) and polydactyly (259 cases, to provide an internal control by a malformation with a strong genetic background) were included. The study was based on a total number of 621,026 deliveries. The effect of radiation exposure (diagnostic x rays administered for medical reasons) on the occurrence of malformations was evaluated in terms of relative risk in a matched-pair case control study aimed at evaluating the association of defects with different types of x-ray examinations received by the mother before or during pregnancy. There was no statistically significant increased risk of malformations to the fetus from pelvis examination of the mother prior to pregnancy. As to examinations received during pregnancy, the risk of malformations after chest x rays was not significant, while for fetal x rays the risk of developing CNS malformations was 2.2 times higher than for those not receiving any examination. For polydactyly, the odds ratio was 1. For abdominal examination of the mother, the relative risk was found to be between 2 and 3 during the various trimesters of pregnancy, but the number of examinations was very small and did not allow any meaningful calculation to be made. In a careful discussion of these findings, the author concluded that the few associations found between congenital CNS defects and diagnostic x-ray examinations could be explained either by chance (no statistical significance) or by the fact that the defect itself was the probable reason for the x-ray examination. It turned out, in fact, that, although the specific reasons for the examinations were not recorded in every case, the great majority of examinations were performed because of clinical suspicion of either maternal or fetal anomaly.

170. Preliminary data have also been reported of a prospective clinical study started in 1967 to provide advice to parents whose children were exposed in early pregnancy to diagnostic x-ray examinations [N2, N7]. In the course of the study, embryological observations after abortion are carried out and the children irradiated in utero are followed up clinically and genetically. Up to 1982, more than 200 cases had been collected and studies performed on 73 children up to 10 years. One publication dealing with this series [N8] provides useful information as to the time at which irradiation occurred, the doses received, chromosomal analyses of 28 irradiated children, and the instances in which spontaneous or induced abortion occurred. But it gives no information as to the number and type of malformations found in the irradiated children.

171. Further data [N11] on the 73 children followed to 10 years showed that, of those exposed in the dose range below 100 mGy, there were two malformations (3.4%) that could not be associated with radiation because exposure occurred before organogenesis. Another malformation (hypospadia and spina bifida occulta) was found on an aborted fetus: here the exposure time might correspond with the time of differentiation of the organs involved. However, the malformations found are not rare among those occurring naturally. In the dose range above 100 mGy, two embryological abnormalities were found at termination of pregnancy. Cytogenetic investigations on 30 exposed children were negative as were other investigations of 21 biochemical genetic markers carried out on a selected group of 19 children. The results of a wide range of pediatric and laboratory investigations appeared, on the whole, to be similarly negative.

172. The search for a possible increased incidence of disturbances in human development among children born in areas of high background has continued. In a paper from China, Lu [L1] reported on a survey of hereditary ophthalmopathies and congenital ophthalmic malformations in the Guangdong province, comparing two areas where the annual cumulative dose equivalents to the whole body were about 2.5 and 1 mSv, respectively. In the area of low background, 66 out of 13,987 children were found to carry eye malformations, to be compared with 99 out of 13,425 children living in the high-background area. The difference was not statistically significant. Two contributions from Ujeno examined the relationships between gonad dose in areas of Japan and the incidence of various conditions. The first paper [U6] dealt with the presence of Down syndrome and visible malformations among subjects born over a three-year period (1979-1981) in 46 prefectures, where the dose rates of natural background radiation varied between

about 0.4 and 0.9 mSv per year. Over 457,397 subjects included in the survey, there were no statistically significant correlations between dose and the incidence of the conditions examined. The second paper [U7] covered 11 prefectures having about the same spread of gonadal dose rates and 1,899,477 births between 1974 and 1976. The following chorionic diseases were examined: hidatidiform mole, malignant hidatidiform mole and chorionepithelioma. Of these, only the first two showed a statistically significant correlation with gonadal dose rate. However, when all diseases were pooled together the regression on dose was found to be non-significant.

173. Evidence of a decreased weight of female offspring born of mothers irradiated (mostly by thorax fluoroscopy) in the course of pregnancy was reported by Ilina [I8], but her findings are not sufficiently documented. Findings of doubtful significance on the incidence of human malformations were also reported in an abstract in 1972 [E1]. Since brain maturation continues beyond birth in man, data on exposure of juveniles are also of interest in a general sense, such as those reported by Glazunov and Tereshchenko [G14] and by Meadows et al. [M3]. Doses of many grays are, however, involved in this experience, and these are far beyond those of interest in the present context. In addition, the relatively low number of cases involved makes them unsuitable for the purpose of risk assessment.

C. CONCLUSIONS

1. Experimental animals

174. The current data on the irradiation of experimental animals during organogenesis have added significant details to the overall picture drawn by UNSCEAR in its 1977 report, but have not strikingly changed its main conclusions. They have confirmed that the effects resulting from irradiation of the mammalian conceptus during the phase of major organogenesis are characteristically teratological. They have also confirmed that such effects, most common in experimental animals, are essentially not seen in man. Teratogenic effects develop during the intra-uterine life as the growth of the organs proceeds, and they persist, if compatible with extra-uterine survival, during post-natal development. Malformations may also be accompanied by growth disturbances of the various structures or of the whole body. The period of main organogenesis of each structure (for example, the skeleton, the brain, the eye) coincides with the period of maximum sensitivity of that particular structure to the induction of malformations, in the sense that the dose needed to produce a given level of incidence of each malformation is lowest when the spurt of cell multiplication occurs in the relevant structure. Increasing the dose either before or after the peak sensitivity period may, however, result in the same type and degree of effect.

175. As organogenesis proceeds at different rates in different animal species, it is to be expected, and it is actually found, that malformations of given structures occur in each species at a characteristic time from fertilization. There is, therefore, a very pronounced dependence of each kind and degree of malformation (for a given acute dose to the embryo) on the animal species and its developmental state at the time of irradiation. Spreading of the same dose over a longer period of time by fractionation or protraction tends to reduce the frequency of occurrence for each class of abnormalities below that expected from an equal exposure at the most sensitive stage.

176. The most recent data have added to the evidence that the form of the dose-effect relationship for each specific type of malformation is generally sigmoid, with the malformation rate per unit dose increasing as a function of dose. This evidence is in agreement with the expectation for effects of the non-stochastic type. It applies to the majority of cases when effects were accurately quantified over a sufficiently large range of doses, which happens particularly in the mouse. It also applies to effects as different as skeletal abnormalities or major malformations, disturbances of brain cell kinetics or pyknosis of cerebellar matrix cells. When irradiations at different developmental ages were compared, as for mouse skeletal malformations, the curvilinear form of the relationship was found to become more pronounced as development proceeds. This is also in agreement with the postulated non-stochasticity of the induction mechanisms.

177. Abnormalities of the mouse skeleton have been particularly well analysed, as they may be scored rather effectively and are fairly easily quantified. Although, in general, sigmoid dose-incidence curves adequately describe their induction pattern, the precise shape of the relationship is different for malformations found in different parts of the skeleton. Accordingly, dose-fractionation and dose-protraction regimes affect the various classes to different degrees, with results that may be difficult to predict in terms of the overall responses for the whole skeleton. To give some estimates of incidence, at the time of maximum sensitivity: in one strain of mouse acute exposures of the order of 5 R were found to triple the spontaneous incidence, although there was no further proportional increase, but rather a decrease, from 5 to 25 R. When the effect was examined in terms of the frequency of animals having a normal skeletal structure, no significant deviation from normal could actually be detected up to at least 25 R. In another strain the quantitative configuration of the axial skeleton appeared to be shifted by an exposure of 12.5 R.

178. Malformations of the CNS have been thoroughly investigated, although in this case the quantitative analysis of dose-response relationships has taken second place to the study of pathogenetic mechanisms. From the overall evidence available in experimental animals, it appears, however, that no effects having clearly pathological connotations have been reported for doses in the brain structures lower than about 0.1 Gy of low-LET radiation. The fairly extensive sets of observations on mice, rats and primates shows that,

depending on the time of pregnancy at irradiation, different structures may be affected, although the pattern appears to be fairly uniform in all species when irradiation takes place at similar stages.

179. In very schematic terms (but with many intermediate forms due to the fact that cells in the developing brain may be at different stages of development at any one time), damage to the developing brain cortex may be induced by a variety of mechanisms. Irradiation at the time of active division of the neuroblasts results in the production of fewer neurons and therefore in a loss of function. Damage to the neuroblasts may to some degree be compensated by a hyperplastic reaction of the glial cell which might restore the size, albeit not the function, of the irradiated structures. During the formation of the cerebral cortex, radiation may interfere with the migration of the neurons (by this time, cells are in a post-mitotic stage). Failure of the cells to reach the appropriate cortex layers causes them to be left behind in the white matter as displaced islands of gray matter, thus disrupting the final brain architecture. Radiation may also disturb the establishment of neuronal connections in neurons that are in the course of migration and are taking up their final position in the cerebral cortex. An impairment of the number of dendritic processes of the neurons, and of their secondary branching, results from irradiation interfering with synaptogenesis.

180. There are indications that each process may be differently affected by irradiation at the various developmental stages, the sensitivity of the neurons decreasing as their maturation proceeds; however, no systematic investigation of this point has been undertaken. In parallel, other processes of maturation, such as the myelination of the fibres or the formation of the tigroid substance in the neurons, may be altered. Glial cells may also be affected in both number and function. Qualitative data on these latter mechanisms are still insufficient and quantitative observations only refer to overall structures and not to specific cell types. In other sections of the CNS, such as the cerebellum, essentially similar effects have been described: however, they take place at times and with mechanisms that are characteristic of these structures.

181. Functional deficits resulting from structural damage in experimental animals have not been studied systematically and so far there has been little attempt to correlate precisely the lesions in given brain structures with the resulting loss of the functions under their control. In rodents, disturbances of conditional reflexes, impairment of learning ability and locomotor damage have been reported after various, but mostly fairly high, doses causing, or expected to cause, gross structural damage. Relatively minor functional deficits induced by irradiation in utero have been shown to be alleviated by stimulation of nervous and behavioural activity post-natally, although the more severe damage induced by the higher doses appears to be largely, if not wholly, irreparable. In primates, impairment of nervous reflexes, behavioural and motor activities, visual acuity and orientation and neuro-muscular co-ordination have been described, but the documentation in this respect is only indicative and therefore insufficient.

2. Human observations

182. In man, teratological effects of the type described in experimental animals are uncommon. As to other abnormalities, a significant step forward has recently been made with a re-evaluation of clinical findings and doses in children exposed in utero during the atomic bombing of Hiroshima and Nagasaki. The latest data show that mental retardation is the most likely type of developmental abnormality to appear in the human species. Severe cases of mental retardation have been included in the study sample. The association between mental deficit and small head size is not very close. In essence, analysis as a function of time shows that the probability of radiation-related mental retardation is essentially zero with exposure before 8 weeks from conception, is maximum with irradiation between 8 and 15 weeks (at the time of rapid neuronal production and migration), and decreases between 16 and 25 weeks when glial cell proliferation and synaptogenesis occur. After 25 weeks and for doses below 1 Gy no case of severe mental retardation has been reported. Analysis as a function of dose shows that the incidence of severe mental retardation is apparently linear for irradiation between 8 and 15 weeks and curvilinear (concave upward) between 16 and 25 weeks. On the assumption that the induction of the effect is linear with dose, the probability of induction per unit absorbed dose at the time of the peak sensitivity is of the order of $4\ 10^{-1}\ Gy^{-1}$ (fetal dose) and of the order of $10^{-1}\ Gy^{-1}$ between 16 and 25 weeks from conception.

183. These findings are important for a number of reasons. First, they emphasize that the most common types of malformation to occur in man are those of the brain cortex, resulting in severe mental deficit. Secondly, they extend to man the notion of a close correlation between the time of most active division of precursor cells of the cortical neurons and the time of maximum sensitivity of the brain cortex to radiation-induced malformations. Thirdly, they show that similar mechanisms of action as those experimentally seen in animals may be operating in the developing human brain, with due allowance for the time scale over which the relevant events occur in different species. Finally, they allow reasonable quantitative estimates of risk to be made for the induction of radiation-induced severe mental deficit in man. While not all aspects of the reported findings may be explained at the present state of knowledge, it is expected that they may have a significant impact in radiation protection in the exposure of pregnant women for occupational or medical reasons. Further developments in respect of functional disturbances of lesser severity than mental retardation may also come from studies currently under way on this cohort of people. Other newly reported observations on radiation-induced malformations in man are much less informative and essentially negative in showing any effect.

IV. THE FETAL PERIOD

184. The 1977 UNSCEAR report [U2] reviewed much information concerning irradiation during the fetal stage of development, pointing out the heterogeneity of the observations. It appeared that lethal effects and growth disturbances had been commonly described for irradiation at high doses, while teratogenic effects became increasingly difficult to document macroscopically as fetal development proceeded. Effects on the eye, the CNS and the gonads were the most commonly described consequences of irradiation during the fetal stage, in addition to a large variety of other effects, e.g., the haematological. The most recent literature has added considerably in some areas, such as the effects on the CNS and on the gonads, but little elsewhere. The effects on the CNS have been considered in III.A, although they actually start to occur at the borderline between the embryonic and the fetal period in man, and extend well into the latter. The present chapter groups the effects on the gonads and on the reproductive system (including external and internal exposure) and all other miscellaneous effects. It should be pointed out that in some experimental series, embryonal as well as fetal exposures are included.

A. EFFECTS ON THE GONADS AND REPRODUCTION

1. The mouse

185. In 1978, following other experiments previously discussed by UNSCEAR [U2], Nash and Sprackling [N1] reported on the reproductive capacity of animals belonging to 13 inbred mouse strains that had been exposed continuously, for a varying number of generations, to ^{60}Co gamma rays. Over the period the experiments lasted, the exposure rate changed between about 7.8 and 0.33 R per 22-hour day. Ancestral and direct exposure values were determined when the animals were removed at weaning from the irradiation chamber and tested for their reproductive performance, using standard tests, to measure percentage fertility, mean litter size and ratio of living to total embryos. Each female (irradiated or control) was classified as sterile or fertile. The latter were killed at the 10th day of pregnancy for scoring of the pregnancy outcome. This included counting, for each litter, the number of resorptions, the number of dead or live embryos, and the percentage of live to dead embryos.

186. The data showed [N1] that for ancestral exposures of 1100 R or greater nearly all females were sterile, but the incidence of sterility at exposures from zero to 200 R was little affected. Response to direct irradiation was found to be more pronounced, with extensive sterility ($> 70\%$) at 200 R or above and no fertile females above 900 R. In the range of exposures below those that induced complete sterility, the overall fertility curves were found to be sigmoid, an observation indicating that this damage is of a cumulative type. Eleven out of the 13 strains studied showed a significant decrease in fertility with increasing ancestral or direct irradiation. Analyses of variance were carried out to detect the presence of linear, quadratic and cubic components in dose-effect relationships, or possible strain differences which might be reflected on total litter size or on the ratio of living to total embryos. In each instance, strain effects were found to be significant. Litter size was more likely to be affected by radiation than the ratio of live to total embryos. Interpretation of these results is made difficult by the fact that the results of similar experiments reviewed previously by UNSCEAR [U2] do not compare in design with this series, so that it is not clear to what extent the differences might be attributable to the different designs or to the methods of scoring and analysis.

187. Adult female mice of the strain A/J received tritiated water by injection on the day of fertilization, so that the tritium content in the body water was brought promptly to levels of about 310, 31 and 3.1 kBq/ml. They were also started at the same time on drinking water containing about 480, 48 and 4.8 kBq per ml of tritium to maintain the initial levels during the entire periods of pregnancy and lactation. Female F_1 offspring thus exposed from conception to 14 days of age were killed at this time for counting the total number of oocytes in serial sections of their ovaries. It was calculated that the whole-body doses accumulated in the 33 days from conception to killing were from 0.79 Gy to 0.0079 Gy at the highest and lowest levels of contamination, respectively. The numbers of primary oocytes were lowered by the following percentages with respect to control: at 0.79 Gy, more than 90%; at 0.079 Gy, 30%; at 0.0079 Gy, 13%. It must be noted that the lowest level of tritium contamination is substantially below those previously reported to cause measurable biological effects in mammals. The dose-effect relationship was said to be exponential and without threshold within the dose interval referred to above, indicating that, in a female mouse exposed continuously from conception to 14 days of post-natal age, the level of tritium that may be expected to result in a 50% loss of the number of germ cells is about 74 kBq per ml body water, corresponding to 0.12 Gy [D8].

188. After reporting that a single injection of 5 MBq tritiated water/g body weight, given to pregnant mice on gestation day 9, reduced the reproductive performance of the offspring [T7], Török and Kistner examined this effect systematically as a function of the activity administered and of the day of administration [T1]. The injection time (a single intraperitoneal administration of 5 MBq/g to the mother, corresponding to a pre-natal dose of about 1.3 Gy) was varied between 5 and 13 days p.c. In addition, three activity levels were tested at this latter time (5, 2.5 and 0.625 MBq/g, corresponding to pre-natal doses of 1.3, 0.6 and 0.2 Gy, approximately). At 2 and 3 months of age the offspring were mated, in single pairs, with control mice of the other sex for 10 days consecutively and then separated. Mice were also set up for histological analysis of the gonads. Fertility was not influenced by treatment on gestation days 5 or 7, but starting from day 9 p.c. in females, and from day 11 p.c. in males, a 50-100% decline by comparison with

controls was found. Administration of tritium on gestation day 13 had the greatest effect. Male fertility was less affected. At 2 months of age, all males and 40% of the females were infertile, while at 3 months female fertility declined and male fertility tended to improve. These observations were broadly in accordance with histological findings. Analysis as a function of dose showed that the fertility of both sexes was affected by doses in excess of 0.5 Gy, while, at a constant dose rate, 3.3 Gy from HTO would be needed to decrease reproductive performance. The late organogenesis stages were found to be the most sensitive under these conditions.

189. Török et al. [T7] reported a significant decrease in the weight of brain and genital organs at 4.5 months in the offspring of NMRI mice given a single intraperitoneal injection of tritiated water (2.6 MBq/g) at 9 days of pregnancy. The number of oocytes in the ovaries was reduced and the seminiferous epithelium of the male mice was considerably damaged. These mice had been found to be fertile at 2 months of age, while at the same age doses twice as large affected the fertility of females but not that of males, and doses four times as large resulted in sterility of both sexes. At 18 months of age, the ovarian tumour incidence of the controls (14%) was increased almost 5-fold (67%) by the administration of about 10 MBq/g of tritiated water. As to effects before or at birth, about 20 MBq/g at 7, 9 or 11 days p.c. resulted in 100% peri-natal mortality, increased resorption and stunting, but negligible incidence of gross external or skeletal malformations. Administration of tritium in excess of 37 MBq/g caused maternal death. In the offspring, damage to the prosencephalon and the gonads stood out generally as the most prominent histological finding.

2. The rat

190. Coffigny and his group [C5] reported on experiments in which pregnant (14 to 21 days p.c.) and new-born rats of up to 2 days of age received a single dose of 1.5 Gy of ^{60}Co gamma radiation. Neo-natal mortality, body weight and adult brain weight were investigated. Among the effects found, germ cell killing was particularly notable. This became the subject of another publication [C7] in which the observations were extended to animals irradiated between 10 days p.c. and 6 days after birth, with the same dose being delivered. When the male animals irradiated in utero reached adulthood, they were studied for testis weight and histological appearance, for the weight of the epididymis and seminal vesicles, and for testosterone plasma concentrations. Testis weight was found to be slightly reduced by irradiation at day 15, but progressively more so at later times, with a maximum between 18 days p.c. and 3 days after birth. A reduced number of spermatogenic cells in the seminiferous tubules accounted for the reduction in mass of the organs. The weight curve of the epididymis followed, in the main, that of the testis but the weight loss of the seminal vesicles was rather moderate. Testosterone secretion was not adversely affected, suggesting that the endocrine function of the testis is, on the whole, rather resistant, as might be expected.

191. The same conclusions were drawn from another study [C6] in which the adrenal activity of male and female rats was tested after 1.5 Gy administered on day 15 of gestation, the time when the hypothalamic nuclei and the pituitary and adrenal glands begin to differentiate. When the irradiated animals reached adulthood, their brain mass was severely decreased, both absolutely and in relation to the whole body mass. The weight of the pituitary and adrenal glands was also reduced absolutely, but not in relation to the whole-body weight. The functional activity of the hypothalamo-pituitary-adrenal axis, as measured by adrenal and plasma corticosterone levels, their diurnal variations, their response to stress or pharmacological challenge were found to be normal, in spite of the gross lesions of the brain and gonads.

192. Reported changes in the fertility of females irradiated during the stage of major organogenesis are scanty. Shapiro [S53] noted the occurrence of sterility in 36% of female animals that had exposures of 50 R at 9 days p.c. Tests conducted by Kakushine and Plodovskaya [K29] showed that 70% of rats exposed to 250 R on day 20 p.c. were sterile.

193. Pietrzak-Flis [P5] investigated the effects of low doses of tritiated water or organically bound tritium ingested chronically, on the number of oocytes in the ovaries of contaminated rats. After adjusting the ^{3}H concentration in water and food to levels that would give similar total ingestion, female rats were exposed starting 5 weeks before mating with a non-exposed male and up to the 21st day of life of their offspring. Female offspring of the F_1 and F_2 generation were sampled at 3 weeks of life for tritium analysis and oocyte counts. There was a close similarity of the doses calculated in different tissues between animals receiving tritiated water or organically bound tritium, although the contribution of the non-exchangeable tritium to the total dose was on average 3 to 7 times higher in animals fed tritiated food than in those drinking tritiated water. A lower (although not statistically significant) number of oocytes was found in contaminated females than in controls. On the basis of some simplifying assumptions, it was calculated that the mean dose to the ovaries was 73 mGy. This led to a 10% loss of oocytes, both in F_1 and in F_2 animals. It appeared, therefore, that the total absorbed dose, rather than the form under which tritium was administered, was the variable that mattered most for oocyte survival.

194. In a subsequent series of experiments [P15], the same author exposed rats continuously to a constant activity of tritium in the drinking water or to organically bound tritium from the time of conception of the F_1 generation through maturity. She calculated that the two groups of animals exposed to tritiated water had a mean absorbed dose rate in the ovaries of 7.25 ± 0.37 and 14.73 ± 0.79 mGy/day, respectively. The group receiving tritium-contaminated food received on average 4.84 ± 0.25 mGy/day. Female offspring were killed when 21 or 71 days old for oocyte counting. The reduction of the number of oocytes in the animals exposed to organically bound tritium was larger (for the same dose rate) than in the animals

exposed to tritiated water, and the dose-rate dependence of the reduction of small oocytes was of an exponential type. The damaging effect of tritium appeared to be higher between birth and 3 weeks of age than between 21 and 71 days of age. The highest sensitivity to tritium irradiation was seen in small oocytes and in oocytes with one complete layer of follicle cells; disappearance of these highly sensitive cells resulted, in turn, in an increased relative frequency of growing and large oocytes.

3. Other animals

195. Fractionated x-ray exposures (0.115 Gy per fraction given twice weekly up to 2 Gy) were administered to 12 female bonnet monkeys either between 48 and 104 days or between 77 and 133 days p.c. Follicle counting in the animals' ovaries was performed between 3 months and 1.6 years of their post-natal life. Animals exposed in the first group had a number of follicles approaching that of non-irradiated controls (about 2000), while the others revealed severe ovarian damage, abortive oogenesis, and reduction of the follicle counts to less than one-third of the normal. No other damage in any organ was found except in the ovary [A5, A6].

196. Data concerning gonadal damage by pre-natal irradiation are also available in the cow [E3]. Cows bearing conceptuses of varying ages from 40 ± 5 days p.c. (when gonadal sex differentiation occurs) to 270 ± 10 days (about 2 weeks before birth) were irradiated once with ^{60}Co gamma rays (300 R), an exposure known to be just below that likely to cause maternal death and gross abnormalities in the product of conception. This resulted in an absorbed dose in the fetal gonads estimated to be about 1 Gy. When female offspring reached about 10 months of age, their ovaries were histologically analysed to count the follicles in different stages of maturation. Primordial follicles were significantly lowered (to 64% of control) for exposure between 70 and 90 days of gestation. Since at this stage the germ cell population is characterized by a high proportion of mitotically active oogonia, it was deduced that this class of cells is the most vulnerable to radiation. Follicular development, however (as shown by counts of growing and vesicular follicles), was apparently unaffected by irradiation at all ages tested. Cows irradiated pre-natally under the same conditions at 80 ± 10 or 130 ± 20 days p.c. had a fertility rate and a reproductive performance that were not different from controls over at least five years of post-natal life and three pregnancies.

4. Interspecies comparisons

197. When Dobson et al. [D25] examined the effect of tritium on fertility by measuring the life-long reproductive capacity of treated mice, they found that reproduction was less affected than germ cell number. For doses causing early oocyte losses of 80-90%, the number of offspring only decreased by 20%. However, initial oocyte deficiencies of about 50% resulted in a premature end of reproductive life. Despite well-known differences between the radiosensitivity of human and mouse oocytes (see annex A), the authors attempted an extrapolation between the two species, using tritium data from monkeys. They concluded that pre-natal irradiation was likely to result in premature menopause in women and could possibly follow "relatively low exposures".

198. Two papers [D9 and E2] attempted some interspecies comparison of gonadal cell sensitivity in utero under internal and external conditions of exposure, respectively. Dobson et al. [D9] exposed mice from conception to 14 days of age to tritium in drinking water and established that the concentration required to cause 50% loss of oocytes under these conditions was about 74 kBq/ml of body water, delivering about 0.0056 Gy/day. Female germ cells of squirrel monkeys required an even lower level (about 19 kBq/ml) for the same end-point. Over the full 153 days of gestation of the species, this level would correspond to a total dose of 0.168 Gy, which might further drop to 0.056 Gy if the sensitivity of the oocytes were confined to the last third of gestation. These data are in sharp contrast with the well-known radioresistance of primary oocytes in monkeys and man to acute x-irradiation exposures. It must be assumed, therefore, that the germ cells in the fetal primate are going through a highly sensitive period of short duration, occurring probably around the middle of the last trimester of gestation in the monkey, at which time doses of less than 0.05 Gy are sufficient to cause 50% killing. It appears that in mice this stage of high sensitivity is maximum between 5 and 19 days post-partum, a time at which susceptibility is also high in respect of the toxicity of the polycyclic aromatic hydrocarbon 3-methylcholanthrene. Similarities between rodent and primates, and between radiation and chemically related sensitivity, suggest that there may also be a highly vulnerable period for the oocytes of the human fetus located approximately at the middle of the third trimester of pregnancy, which might represent a special hazard in relation to chronic exposure.

199. Erickson [E2] irradiated continuously, throughout gestation, mice, rats and guinea pigs at rates of 0.01 to 0.03 Gy/23 h day. Pigs were also irradiated continuously for 108 days of gestation at 0.0025 Gy/23 h day. In all species, the gonads were sampled at birth or at 6 days of age and the numbers of germ cells per organ were estimated as a function of dose. The D_0 and extrapolation numbers (n) were calculated to be as follows, on the basis of a single-hit multi-target model:

		D_0(Gy)	n
Pig	male	0.28	0.8
	female	0.27	3.2
Rat	male	2.75	0.3
	female	1.59	0.8

The low value of the extrapolation number suggests that in both males and females (but especially in the former) the germ cell response is dominated by single events. The value of 0.3 for the extrapolation number in male rats could also be attributed to heterogeneity

in the composition of the irradiated cell population with respect to their sensitivity to radiation exposure.

200. After irradiation at 0.01 Gy/day throughout pregnancy, the germ cell counts were reduced to the following percentage of the controls in the species tested:

	Total dose (Gy)	*Percent of control*	
		Male	*Female*
Pig	1.08	1	5
Guinea pig	0.62	41	71
Rat	0.21	50	90
Mouse	0.20	71	87

These data show that the pig is by far the most sensitive species with respect to continuous irradiation during the pre-natal period, but this conclusion does not take into account the large interspecies differences in the length of the developmental period of the male and female germ cells. The paper [E2] provides useful data for the relevant comparisons, collated from other reports and from original work (references are to be found in publication [E2]). The data are summarized in Table 11.

201. Erickson's data [E2] show that in spite of the large variation of the kinetics of primitive germ stem cells in the various species, the pig remains the most sensitive species even when total doses (rather than dose rates) are used as the basis for comparison. The data also show that male germ cells are more readily killed by protracted exposure than female ones as a result of both their kinetics and of the protracted period of vulnerability. However, the data do not offer a satisfactory account of the relatively lesser susceptibility to acute than to protracted regimes of irradiation, because they may not entirely explain how the refractoriness to acute exposure of a significant segment of the primitive germ cell population might be overcome by chronic exposure. The differences shown in Table 11, and various arguments put forward in the paper, point to the need to extend consideration of this subject and of the relevant interspecies comparisons much more than has been the case so far. Sensitivity to mitotic delay, in addition to cell killing, will have to be explored further and in a systematic manner in future work.

B. OTHER MISCELLANEOUS EFFECTS

202. A number of other miscellaneous effects have also been described. Thus, for example, Swiss albino mice irradiated in utero at days 11 1/4, 14 1/4, 16 1/4 and 18 1/4 p.c. with ^{60}Co gamma rays (150 or 250 R) showed a dose-related decrease in the number of goblet cells in the crypts (and to some extent also in the villi) of the gut at 1 day and 1, 2, 4 and 6 weeks post-partum [M46]. Chajka and Lobko [C16] studied rats irradiated with x rays at 12-14 days of fetal age and investigated from day 13 to 22 the formation of the paramesonephral tracts, describing in detail disturbances in the development of the vagina.

203. Gerber and Maes [G4] investigated the radiosensitivity of blood-forming stem cells in the bone marrow and the spleen during the fetal hepatic period (18 days p.c.) and during the post-natal and adult phases of haematopoiesis. On the basis of their capacity to produce spleen colonies in heavily irradiated transplanted mice, the stem cells isolated from the fetal liver had a D_0 of 1.29 Gy, as compared with 0.70 Gy and 0.86 Gy for the spleen and marrow cells of adult animals, respectively. Together with a decrease in the D_0 values after weaning, the percentage of cells in the S-phase and the rate of division of transplanted cells in the irradiated hosts also decreased. There was, however, no significant difference in the D_0 values between haemopoietic cells in all phases of the cell cycle and the ^{3}H-thymidine-sensitive cells in the S phase.

204. Weinberg and her group [W24, W25] described disturbances of B6D2F1 mouse and HA mouse [W33] haematopoiesis found at various times of pre- and post-natal life and induced by irradiation (0.5-3.0 Gy, ^{60}Co) of the animals at 10.5 days p.c. They consisted essentially of disruption of fetal liver cellularity, morphology and haematopoietic cell concentration extending to 14 weeks of extra-uterine age. The same authors also examined haematopoietic alterations in the beagle dog irradiated during mid-gestation at 33 days p.c. (an age corresponding approximately to that of the 10.5 day-old mouse) with 0.9 Gy from ^{60}Co gamma irradiation [W21]. Various blood indices were investigated at 42-59 days of gestation. Differences between irradiated and non-irradiated animals included a decrease of nucleated cell counts in the peripheral blood between 44 and 49 days, followed by an overshoot at later times; changes in the size and haematopoietic activity of the spleen; an overall decrease in haematopoietic functions of the fetal liver; and an increased activity of the bone marrow. The experiments left undecided whether the observed changes might be due to direct radiation damage on the migrating stem cells or to a radiation-induced defect of the intercellular interactions in the microenvironments that regulate haematopoiesis.

205. In other experiments [M2], pregnant sows were exposed to whole-body gamma radiation at rates of 1.5, 3, 9, or 20 R per day (exposures actually lasted 22-23 hours per day) for 108 days of their average gestation period of 112 days. After 3 weeks of continuous irradiation, all groups had depressed leukocyte counts which were generally related to the exposure rate. Counts remained low throughout the whole irradiation period and they did not completely recover even 3 weeks after discontinuation of exposure in post-natal life. As to the effects on the piglets, those that had been exposed to 3.2 R/day showed no effect on the leukocyte system, and those in the 7 R/day group had a moderate lymphocytopenia at birth which rapidly disappeared. This differential effect between adult and fetal animals took place in spite of the fact that the fetal leukocyte system is functionally mature during the last 5 weeks before birth.

206. To facilitate extrapolation of the above data, it should be recalled that in man the first blood cells and blood vessels are found in the extra-embryonic mesenchyme at about 18 days from conception. This

first period of haematopoiesis is succeeded and gradually replaced by blood formation within the embryo, chiefly in the mesenchyme of the liver, during the second month of development. At still later stages, starting from the 11th week on, a third period of haematopoiesis commences in the bone marrow and the lymph nodes. There is considerable overlapping between the three periods and it is only the last type of haematopoiesis that persists normally in adult life [H27]. Data obtained directly on human samples are, however, very rare. In one paper, no post-natal abnormality appeared to be associated with fetal exposure in the range of 0.01-0.03 Gy of 498 children born during 1963-1977 and examined when 5-19 years old. There were after birth 5 deaths due to accidents or acute infections, but the 493 survivors were normal in respect to stature, peripheral blood counts, mental development and, in females, menarche [L3]. In contrast, 25 children exposed in utero to diagnostic x irradiation after the 8th month of intra-uterine life to estimated doses of about 0.03-0.2 Gy showed significantly lowered leukocyte counts at 2 days and 1 year after birth [Z4].

207. Konermann [K33] reported a post-natal study of the liver of mice in which he analysed, as a function of dose and time, the growth response of the organ in animals that had been irradiated in utero. The animals were exposed to between 10 and 60 R/day during different periods of their pre-natal life, as follows: blastogenesis (1-5 days p.c.); organogenesis (6-13 days); fetogenesis (14-18 days); early embryogenesis (6-10 days); and late embryogenesis (11-15 days). In addition to weight, the content of DNA and, the ^{3}H-thymidine uptake into liver cells were followed for up to 11 weeks after birth. All irradiated animals showed a periodical increase of the proliferative liver growth, especially between the end of the first and the beginning of the second post-natal week. This proliferation decreased with the age of the irradiated conceptuses, from a clear over-compensation during blastogenesis to poor response during fetogenesis. There was also a dose dependence of the effect which (during organogenesis) was biphasic, in the sense that compensatory proliferation was small at 10 R/day, maximum at 40 R/day, and declining at 60 R/day. Overall, these responses were interpreted as attempts by the organ to attain a pre-determined normal size, but appeared to differ in many respects from the hypertrophic regenerative changes induced by post-natal liver damage. First, pre-natally induced proliferation affects, at the same time and to similar extent, both the haematopoietic and the parenchymal cells of the fetal liver, which are considered to be functionally independent components of the organ. Secondly, this compensatory growth is maximum at similar developmental stages and the maximum is not greatly dependent on the radiation dose and on the age at irradiation. Finally, radiation-induced growth stimulation of the liver appears to take place in the absence of any change or even in the presence of a decreased overall body weight.

208. About 100 beagle dogs exposed to ^{60}Co gamma radiation (270-435 R) at 55 days p.c., or at 2 days post-natal age, were examined at 7 days and at 2 and 4 years of age for effects of radiation on the kidney [J2]. Morphometric studies showed an arrest of development and maturation of the organ. For pre-natal exposure, the density of mature glomeruli was 45% less than in non-irradiated controls, while for post-natal exposure the density was only 24% less. At 2 and 4 years, a smaller reduction was observed in the same direction (37 and 32%, respectively). Glomeruli that were mature at the time of exposure underwent progressive sclerosis, characterized by proliferation of the mesangial cells and increase of the mesangial matrix, leading to a reduction of the lumen of the capillaries and the complete obliteration of many glomeruli. Thus, about 21% of the animals died from chronic kidney failure before 4 years of age: this condition was more common in male (19/58) than in female dogs (2/41), pointing to a major sex difference of unexplained origin.

209. It was also reported in the same series of experiments [L2] that an exposure of 100 R at 55 days p.c. resulted in about 80% of the animals having missing teeth, compared with about 57 and 26% in animals irradiated at 2 days p.c. or in control dogs. The upper and lower premolars were the teeth most frequently absent.

210. Developmental toxicity evaluations do not often include functional deficits, but are mostly limited to gross effects such as embryo lethality, malformations and growth disturbances. In a methodological contribution, Andrew [A7] proposed the study of developmental enzyme patterns as a means of directly evaluating the integrity of metabolic competence of developing organ systems. The full complement of enzyme activities is usually developed in mammals in a characteristic sequence: alterations from this normal pattern may occur at three periods, namely, the late fetal, early neo-natal and weaning times. The author argued that qualitative or quantitative changes in the patterns of development of one or more key enzymes in a tissue might be taken to indicate developmental toxicity. Some contributions reported along those lines in respect to radiation toxicity were already discussed in the 1977 UNSCEAR report [U2].

211. Some papers from the USSR dealt with disturbances of the enzyme levels in the liver of rats irradiated in utero. The first [S51] reported a reproducible decrease of the glucose-6-phosphatase activity observed in 21-day old fetuses that had been irradiated with 2 Gy on day 9 p.c. As was later shown, this decrease may be removed by the administration of exogenous thyroxine [K25]. The second paper [S52] reported increased tyrosine aminotransferase activity in new-born rats irradiated with 0.5 Gy on the 9th day after conception. This effect correlates with the status of the cyclic-AMP system. It was proposed that the above effects in the liver of the rat at term, following irradiation during early organogenesis, may result from changes in the amount of inductors [K25].

212. Brain weight and enzymatic activities were also investigated by Weber et al. [W8]. In an attempt to develop more sensitive end-points of damage by pre-natal irradiation, NMRI mice received a range of

doses (x rays, 0.24 to 1.9 Gy) on the 12th day of gestation. They were then sampled at various times between birth and 64 days of age to investigate the weight of their brain and the levels of acetylcholinesterase and of Na,K-ATPase in the organ. Brain weight was related to the enzyme activities in a non-linear fashion: for both enzymes there was a relatively flat dependence up to a brain weight of 350 mg (corresponding to a control age of about 17 days) and a steeper relationship as the brain weight increased. The weight of the brain was reduced in increasing proportion to dose to reach about 40% of control after the highest dose. The data were interpreted to show that, in spite of this reduction, the developmental maturation of the organ was not retarded.

C. CONCLUSIONS

213. As the growth of the anatomical structures established during the phase of major organogenesis proceeds during fetal development, fewer teratological malformations are being produced for the same radiation dose. However, more subtle changes, often documented at the microscopic level, and growth defects of the developing structures, are commonly described. During fetal development, the periods of most active growth of anatomical structures are also, in general, those that are most susceptible to radiation action. Approximate dose-effect relationships may be established for both external and internal exposures in the best documented series.

214. Among the many effects produced by external irradiation of fetal animals, those on the developing gonads have been particularly well described for a variety of animal species to a reasonable degree of quantitative detail. In the mouse, studies were made of both the inactivation of gonadal cells and the ensuing loss of reproductive performance when the animals reached sexual maturity. For external irradiation, female fertility was not appreciably affected up to 200 R of ancestral irradiation, but sterility increased conspicuously when the same exposure was delivered directly to a developing animal. Pronounced sigmoid relationships appeared to prevail for the impairment of reproductive capacity, and a good degree of inter-strain variability has been documented.

215. In the mouse, experiments on internal tritium irradiation were reported after long-term exposures from conception up to 14 days of age, as well as for single treatments delivered at various times during development. Under the first condition of exposure, the activity of tritiated water needed to cause a 50% loss of oocytes in the female gonads was estimated at about 74 kBq per ml of body water (corresponding to a uniform whole-body dose of about 0.12 Gy) with an apparently exponential relationship between absorbed dose and oocyte killing. For single injections, the late stages of organogenesis were the most sensitive in respect to loss of fertility. In both sexes, fertility was affected by doses in excess of 0.5 Gy, but male gonads tended to recover at longer times from treatment, while female fertility continued to decline.

216. In the rat, external radiation exposure resulting in doses of 1.5 Gy caused measurable male germ cell killing, which persisted long after birth without evidence of any adverse effect in the endocrine function of the gonads, or of the hypothalamo-pituitary-adrenal axis. Tritium, given as tritiated water or as organically bound tritium over many generations to developing animals, caused a loss of oocytes in the female gonads for doses of the order of 0.07 Gy. In the bonnet monkey, fractionated exposures up to a total of 2 Gy for periods of about 50 days during the latter part of pregnancy resulted in a consistent loss of oocytes, which persisted into adult life. The same is true for cows irradiated in utero at doses of 1 Gy during the phase of active mitotic division of the oogonia at 70-90 days p.c. However, there was no evidence in these animals that fertility and reproductive performance would be affected, at least over the initial period of their reproductive life.

217. Valuable interspecies comparisons of the sensitivity of the developing gonads under internal and external radiation exposure have also become available. For one species of monkey, there is evidence that the germ cells go through a highly sensitive period of short duration around the middle of the last trimester of gestation when doses from tritiated water, of the order of 0.05 Gy, might cause one-half of the oocytes to die. For external irradiation, comparisons of various species showed the existence of a wide scale of sensitivity values. It is clear that the intrinsic radiosensitivity of the gonadal cells, together with their kinetic characteristics, determine in the various species the apparent sensitivity of the irradiated germ cells. It is necessary, therefore, to make comparisons between species, not only in respect of dose-rate, but also in terms of total dose received by the germ cells during the whole sensitivity period. When this is done, paradoxical effects such as an apparent higher response of the developing testis to protracted than to acute irradiation are found. This is indicative of the existence of phases of high sensitivity in the development of the male gonadal cells, an effect known to occur also in the adult testis. Much remains to be done to resolve and clarify these inter-species differences upon fetal irradiation.

218. A variety of miscellaneous effects have also been described for irradiation of fetal stages. The blood-forming system has been closely investigated, but detailed analysis of the radiation action is made difficult by the transition from the hepatic to the adult type of haematopoiesis which occurs before birth. Acute doses of a few tenths of Gy, or continuous exposures to a few roentgen per day, are necessary to elicit consistent changes in the blood-forming organs and the peripheral blood of the fetus. The persistence of the changes observed throughout the post-natal ages, and their significance in respect of normal haematopoiesis, are difficult to evaluate. Time- and dose-related hyperplastic reactions of the irradiated fetal liver, both in its haematopoietic and parenchymal component, have also been described. They may generally be interpreted as compensatory regeneration stimulated by the radiation-induced killing of cells. But they have a distinctly different character from the

regeneration phenomena induced by post-natal liver damage. Kidney lesions may also be induced by irradiation during the fetal stages. This damage primarily affects the capillaries and is mostly manifested by glomerulosclerosis resulting in chronic kidney failure during extra-uterine life.

219. Finally, attention should be drawn to recent attempts to develop tests of toxicity induced in irradiated fetal organs by measuring the level of enzyme activity and the development of the characteristic adult enzyme pattern. These tests are different from macroscopic or microscopic methods for scoring of malformations because they aim at documenting functional, rather than morphological, derangements from normal. While they may turn out to provide more sensitive techniques of assaying the radiation-induced damage in utero, their full methodological potential, and their relevance to clinically documented detriment, remain to be established.

V. INTERNAL IRRADIATION

220. The information available to UNSCEAR up to 1977 was insufficient for statements of general validity regarding the effect of radioisotopes taken in by the mother and irradiating conceptus. Given the chronic nature of the exposure, any evaluation of the effects and risks must be based on a precise knowledge of the dose accumulated at each particular stage in any given developmental structure, but the achievement of such a task for all nuclides and all possible conditions of administration is still far away. UNSCEAR identified a number of variables which may interact in the production of any end-effect. They are: the physical and biological characteristics of the nuclide; its chemical form; the route and the dosage schedule of administration; the kinetics of the radioactive material in the mother-fetus complex; the developmental stage at the time of treatment in relation to periods of maximum sensitivity; the species and age-related variables; and, finally, the role of maternal exposure, particularly at high doses.

221. Information in this field that has come to the attention of UNSCEAR since about 1977 has added somewhat to the previous knowledge. However, this is hardly significant compared with what is required to produce widespread generalizations relating the level of uptake of radioactive substances by the mother to the probability of effects that might be expected in the conceptus. The following paragraphs summarize the main new findings regarding the various nuclides.

222. The methodologies for the study of placental transfer have been reviewed [S16]. Moreover, factors influencing the transfer of non-radioactive substances injected into the mother have been discussed by Klinkmüller and Nau [K7] who have compiled a review of the transplacental passage of drugs through experimental observations carried out on aborted children. Some information on the placental transfer of proteins in the human species is also to be found in a paper by Dancis et al. [D2].

223. Stieve [S66, S68], Roedler [R27] and Roedler et al. [R28] reviewed experimental literature (see also a report by Ahrens et al. [A11] for a bibliographic list) and discussed the general aspects of exchange and transfer mechanisms of radioactive substances between the mother and the conceptus as they vary with the time of intra-uterine development, and the various physical and biological factors governing such transfers. It is obvious from these analyses that the need to determine experimentally the transfer coefficients and their inter-species variability prevents any straightforward extrapolation from animal to man. Examples drawn from experiments using tritium, iodine, calcium and caesium were given to illustrate the complexity of the problems involved in assessing the rate and amount of transferred radionuclides. Transplacental transfer of iron in animals and man have also been discussed [F9, T14]. Data referring specifically to tritium have been provided by Moskalev et al. [M15], and a compilation of data for the radioactive nuclides of iodine, iron, strontium and caesium has also been made available to UNSCEAR [S81].

224. Ovcharenko [O10] examined the transfer of radioactive substances from the mother to the newborn through lactation and concluded that the rate of transfer is governed by a number of factors similar to those affecting the passage across the placental barrier. In particular, he reported on an inverse relationship between the rate of transfer and the mass number of the radionuclides, in addition to a profound influence of the animal species and the body weight of the offspring. He pointed out, however, that the main factor in the amount of radioactive material passed on to the offspring through the milk is the level of radionuclide in the mother's blood.

A. EXPERIMENTAL DATA

1. Tritium

225. Yamada et al. [Y1] investigated the response of pre-implantation $BC3F_1$ mouse embryos to chronic exposures of both tritiated water or ^{60}Co gamma rays. Embryos were cultured in vitro in chemically defined media containing tritium oxide at activity levels from 3.7 to 74 MBq/ml. Taking the progression of 50% of the embryos to the blastocyst stage as the end-point for survival, the activity needed to achieve this end-point (LD_{50}) was found to be about 4.9, 8.5 and 16 MBq/ml for pronuclear, early 2-cell and late 2-cell embryos, respectively. In comparison with the chronic ^{60}Co treatment, the RBE of the beta radiation of tritiated water was calculated to be between 1.0 and 1.7. On the basis of other experiments on the induction of chromosomal aberrations in mouse zygotes fertilized in vitro, the RBE values for tritium were reported to be 1.8 and 1.5, relative to ^{60}Co gamma rays and to x rays, respectively [M56].

226. In another study [S18], an attempt was made to compare the outcome of tritiated water and tritiated thymidine treatments on pre-implantation mouse embryos cultured in vitro. Also in this study, the end-

point scored was the ability of the embryos to reach the blastocyst stage; tritiated thymidine was about 1000 times more effective than tritiated water in inhibiting blastocyst formation. The treatment with tritiated water was particularly effective in inhibiting the late stages of blastulation, while thymidine blocked the rapid cleavage stages. Thymidine was concentrated in the nuclei through DNA incorporation by a factor of about 1000. In very general terms, and under specific assumptions applying to the system analysed, it was concluded that there was a reasonable agreement between the dose per cell nucleus and the inhibition of blastocyst development, such that about 19 kBq/ml of tritiated thymidine corresponded to about 19 MBq/ml of tritiated water. Observations on the development of pre-implantation mouse embryos, and a discussion of effects in relation to path length and energies of the electrons emitted by ^{3}H and ^{35}S, are to be found in a paper by McQueen [M1].

227. Spindle and Pedersen [S74] investigated the effect of ^{3}H-thymidine on the development of early mouse embryos by counting the number of embryos forming blastocysts, trophoblast outgrowth, inner cell masses and differentiated primary endoderm and ectoderm. Embryos were cultured from the 2-cell stage continuously for 8 days in the presence of ^{3}H-thymidine in various concentrations. Concentrations as low as 7.4 Bq/ml reduced the percentage of embryos forming the two differentiated primary layers. At 370 Bq/ml, the embryos developing to the three post-blastocyst end-points were also reduced. Exposure of the embryos for 1 day at various developmental stages produced effects that were less severe than for continuous exposure for 3 or 8 days. The sensitivity of the embryos differed with stage: the high sensitivity of the inner cell mass to ^{3}H-thymidine, while persisting through the late blastocyst stage, declined progressively thereafter. Autoradiography experiments showed that the radiosensitivity changes of the embryos or of the inner cell mass were generally related to their capacity for incorporation of thymidine into the cell DNA.

228. Yamada and Yukawa [Y4] fertilized BC3F1 mouse oocytes in vivo or in vitro and then cultured the embryos in vitro up to the stage of blastocyst in the presence of chronic irradiation from tritiated water or thymidine, or under acute x-ray exposure in the range of 10-600 R. Taking the development of the embryo to the blastocyst stage as the end-point, the sensitivity of the embryos to the β radiation of tritiated water was confirmed as in Yamada et al. [Y1], while the LD_{50} for tritiated thymidine was given as about 0.63 and 3.96 kBq/ml for pronuclear and late 2-cell embryos, respectively. The highest sensitivity to killing by acute x irradiation occurred at the beginning of the pronuclear formation.

229. Some papers dealt with tritium uptake and turnover in pregnant and fetal animals. Saito et al. [S34] exposed pregnant mice of the Heiligenberger strain to tritium in drinking water as methyl-^{3}H-thymidine at the level of 18.5 kBq/ml. The new-born animals were either nursed by their mothers that continued to drink the tritiated thymidine, or by foster mothers drinking non-radioactive water. The authors measured in the offspring, at various ages, the incorporation of tritium into the small molecular components of the acid-soluble fraction, lipid, RNA, DNA and proteins. At the time of birth, the specific activity of the isotope in the DNA was highest in the heart and lowest in the thymus. Soon after birth, the total radioactivity per gram of tissue declined with a half-life of 2.5-2.9 days in the spleen, liver, intestine, stomach, thymus, lungs, kidney, heart and brain in the mice nursed by the non-contaminated mothers. By about 2 weeks post-partum, a slower component of tritium elimination was emerging, mainly due to the DNA-bound isotope. On the assumption that the total and DNA-bound activity is uniformly distributed in individual organs and tissues, the cumulative absorbed doses in various organs were worked out for the first 4 weeks after birth. These doses were found to be highest in the spleen (1.15 mGy) and lowest in the brain tissue (0.13 mGy). The tritium ingestion from milk in suckling animals was found to be a rather minor source for dose accumulation in the DNA-bound tritium of the cell nuclei of various organs. Similar data were also presented in another paper by the same group [S35].

230. Lambert and Phipps [L7] infused SAS/4 mice continuously during pregnancy at four activity levels, such that the new-borns would be contaminated with organically bound tritium in concentrations of 10.4-130 MBq/kg body weight. Total doses in utero were calculated to be in the four injection groups 0.15, 0.4, 0.76 and 1.72 Gy. The tritium content of various organs was measured in the mice at frequent intervals up to about 2 months of age, and again when the animals died. Full pathological investigations were performed. Early effects were seen, such as reduction of litter size, changes of the sex ratio, and neo-natal mortality, particularly at the two highest activity concentrations. These effects were judged to be more significant overall than late ones, such as a reduction of the long-term survival time which appeared in the highest activity group. There was an indication of an increased incidence of neoplastic and non-neoplastic lesions, possibly related to the intra-uterine dose; however, the significance of the various causes of death could not be judged without a full statistical analysis, which was not performed.

231. Wang and Zhao [W34] performed an experiment in which the effectiveness of β radiation from tritiated water and gamma rays from ^{137}Cs was compared in respect to their capacity to reduce cerebral development in Wistar rats. Females were injected with HTO in various concentrations on the first day of pregnancy and provided from then on with tritiated drinking water, so as to maintain the tritium concentration in body water approximately constant throughout pregnancy. The intra-uterine dose rates in the five groups of animals examined were estimated to be 0, 0.005, 0.012, 0.02 and 0.038 Gy per day. Other pregnant females were irradiated during 22 hours per day throughout gestation with a ^{137}Cs source at 7 dose rates between zero and 0.12 Gy per day. There was a good negative correlation between the size of the cerebral mass at birth and the cumulative radiation dose in the two series. Approximately the same

reduction of the brain weight was produced by 0.22 Gy of β radiation delivered over the whole pregnancy as by 0.47 Gy of gamma radiation (RBE about 2.14). At higher total cumulative doses (0.84 Gy and 1.3 Gy, respectively) the RBE was found to be lower (1.55). A significant decrease of the whole-body weight at birth was only found for cumulative gamma doses of 1.76 Gy or higher. No dose dependence was seen at lower dose levels.

232. In experiments by Lyaginskaya [L9], rats were given single intraperitoneal injections of tritium oxide (33 MBq/g) on days 4, 11 and 17 of pregnancy. This treatment was said to have resulted in fetal exposures of about 4.12, 3.00 and 2.50 Gy, respectively, by the end of the pre-natal life and 412, 420 and 478 Gy by the time of weaning. The descendants of three successive generations of the injected animals showed a reduction of life span and an increased frequency of tumours. The average life span in the various groups was as follows:

	Life span (days)		
Treatment time	*First generation*	*Second generation*	*Third generation*
4th day	378	394	280
11th day	556	705	288
17th day	705	735	618
Controls	910	1060	1020

Thus, the greatest reduction of life span was shown by the third-generation descendants and by the offspring of animals treated early in pregnancy. The overall tumour incidence increased in the offspring from the first to the third generation and was again highest in the offspring of animals injected on day 4.

233. Gerber and Maes [G3] raised pregnant BALB/c mice on food enriched with tritiated thymidine from the day of conception until delivery, for a total tritium intake of 4.8 MBq. At birth, and again at 4 different times up to 109 days of age, the tritium content was measured in the DNA, protein and supernatant fraction of various parenchymal organs. It was found that a total of 2.0-2.5% of the total activity in the neonatal mouse was incorporated into the DNA, while a slightly larger amount entered the proteins and lipids together. Most organs showed two metabolic components of the DNA, one with a half-life of the order of 10-20 days and another (amounting to 10-30% of the total activity incorporated into DNA) with a very slow turnover, of the order of 100 days.

234. Data along similar lines were also reported in the pig, after exposure of pregnant sows to tritiated water (about 19 and 55 MBq/liter) during pregnancy and for 43 days after birth, up to a total of 120 days [V7]. Some of the new-born pigs were left with the mother and the others were foster-fed by non-contaminated sows in order to follow the tritium oxide and organic tritium in different organs. This allowed the study of the loss of activity after birth, the metabolism of tritium under conditions of continuing exposure and the importance of uptake through milk. The turnover time of tritium oxide in the adult sow was found to be about 10 days, that in younger pigs about 8 days. There were, in addition, components of slow turnover, amounting to less than 5% of the total. At equilibrium, the ratio of specific activity of tritium oxide in organic matter to that in tritiated water ingested was about 0.7 for body water in adult and new-born pigs and about 0.14 for organic tritium in most tissues. The ratio was higher (0.22) in the brain of the new-borns than in other tissues. The turnover times, in days, for organically bound tritium were as follows: brain, 59; muscle, 28; kidney, spleen and pancreas, 22; liver and intestine, 17. It was estimated, in conclusion, that the contribution to the integral tissue dose due to organic tritium was between 0.3 and 0.7 that from tritium oxide alone, although in the brain this contribution may equal or even exceed that from tritium oxide.

235. Carsten [C15] described a broadly based programme designed to evaluate the somatic and genetic hazards of continuous exposure to tritiated water for two generations in the mouse. The levels used were between about 11 and 110 kBq of tritium per ml of drinking water and the relative biological efficiency was obtained by comparing the effectiveness of such a treatment with that from continuous exposure to ^{137}Cs gamma. The end-points for comparison were dominant lethal mutations, chromosomal aberrations in the cells of regenerating liver, increased sister chromatid exchanges in bone marrow cells and changes in the bone marrow cell and stem-cell numbers. The RBE for HTO ingestion for all these effects was assessed to be between 1 and 2, compared with external gamma exposure.

236. A series of papers dealing with the effects of chronic exposures to tritium on brain development has been published by Zamenhof et al. [V2, Z1, Z2]. Female rats (F_0) were given tritiated drinking water (110 kBq/ml) from 60 days of age until and throughout pregnancy [Z1]. The maximum concentration of radioactivity in these animals was reached at 30 days and in the blood at 42 days after the beginning of the treatment. Under these conditions, the average dose to the cell nuclei was estimated to be 0.0078 Gy per day. The consumption of water and food and the changes of body weight before and during pregnancy in these animals did not differ from non-contaminated controls. The course of pregnancy and time of delivery were also normal. The highest specific activity in the newborn animals (F_1) was found in the nucleic acid fraction, but the bulk of radioactivity was contained in body fluids. These animals showed no sign of radiation-induced effects. The weight of the new-born animals was normal, but 60% of them had haematomas, oedemas, and subdural haemorrhages, which disappeared subsequently by the time they reached 30 days of age. No haematological changes were observed as a function of age, the only exception being a significant decrease of alkaline phosphatase. In spite of a normal brain weight, these animals had a significantly low content of DNA, protein and protein/DNA ratio in this organ. The administration of tritiated water was continued in the F_1 animals throughout weaning, adolescence, and subsequent pregnancies in the F_3, F_4 and F_5 generations. Analysis of cerebral damage in F_3-F_5 rats showed that this was not more evident than in F_2, i.e., that the effects of tritium were

not cumulative over the generations. There were, on the contrary, indications that brain damage might be less pronounced.

237. The same scheme of tritium administration was followed in other experiments by the same group [Z2]. The brains of the irradiated animals were studied at 30 and 120 days post-natally within each generation. Significant decreases were reported in weight, DNA and protein contents of some parts of the brain and the most pronouced effects were found in the diencephalon. Protein losses were more pronounced than those of DNA and the effects were overall more conspicuous at 30 than at 120 days and in the fifth than in previous generations. There was an attempt to explain these phenomena by considering the periods of maximum sensitivity of the brain structures involved, possible effects on the mothers in each generation and the capacity for repair following the radiation-induced damage to nucleic acids.

238. In other experiments [V2], female rats of 60 days of age were started on tritiated water (110 kBq/ml) and killed when 90 days old. The ovaries were excised and the oocytes removed by puncturing the follicles. Examination of the chromosomes in these cells showed that their rate of maturation was not affected by the presence of tritium. When the offspring of these females were examined at birth, the number of cells in the cerebrum was only slightly decreased as compared to controls. Other rats were exposed to tritium from 30 days before mating to 6 days after mating. This treatment was found to depress the decidual response. The offspring of this group of animals had a significantly lowered number of cerebral cells at birth. This effect was even more pronounced when exposure was continued throughout the pregnancy.

239. Finally, Radwan [R1] carried out a comparison of chronic exposure to tritiated water or organically bound tritium to study developmental (startle response, righting reflex, age at eye opening) and behavioural (diurnal and nocturnal locomotor activities) effects. Wistar rats received tritium at a concentration of 37 kBq/ml in drinking water, or at about 48 kBq/g in food, or no tritium at all for 2 generations. From the overall results, it was concluded that, for the same activity level, continuous exposure to the organically bound tritium was more damaging than that to tritiated water, despite the higher cumulative doses delivered to the brain of the animals by means of drinking water. This may mean that the higher tritium incorporation into the organic fraction of the brain after exposure to the tritiated food is responsible for the effects observed.

2. Radioactive nuclides of iodine

240. Several reports have become available concerning thyroidal exposure during developmental stages. One of them [B6] concerns the metabolism of ^{131}I in guinea-pigs during the last two weeks of gestation. The animals were killed at various times (from 5 hours to 9 days) after injection, and the blood, amniotic fluid, thyroid glands and other tissues were sampled. Over the time indicated, the concentration of the isotope was consistently less in the blood of the mother than in that of the fetus. Moreover, concentration in the fetus was less than that in the amniotic fluid. Peak concentrations of ^{131}I in the thyroids of both the mothers and the fetuses were found at 3 days post-injection. In the fetal thyroid (per gram of tissue) the concentration was 3 times higher. The biological half-life of the nuclide in the thyroid gland was about 3 days in the fetus and about 11 days in the mother. Between 7 and 9 days post-injection, the ^{131}I in the maternal thyroid increased, and this phenomenon was interpreted to show a recycling of iodine within the pregnant animals.

241. Another group analysed the kinetics of diaplacental transfer of ^{131}I in rats fed either standard or iodine-deficient diets [S69]. Female animals were started on diets containing 41 or 320 ng of iodine per gram of food a few days after their birth. When they became pregnant, they received intravenously (on each gestation day from 17 to 20) 0.74 MBq of ^{131}I and were killed at various times (up to 24 hours) from injection. Five fetuses per female were counted for radioactivity in toto and another 5 were dissected for measurement of radioactivity in various tissues, as well as in the blood and the amniotic fluid. The ^{131}I activity concentration per gram of tissue in the fetal thyroid rose by a factor of over 1000 between day 17 and 20 of fetal age. By day 19, 35% of the radioiodine in the fetus was in the thyroid. The concentration in the fetal thyroid never exceeded that of the mother's, at least up to day 20, and it appeared to decrease from day 19 to 20. The uptake of the nuclide in the thyroid of the mothers fed the iodine-deficient diet was much higher and much more rapid than in that of the mothers kept on the standard diet. As to the uptake in the fetal thyroid, it was found to be higher at 2 hours in the offspring of the iodine deficient mothers, but by 8 and 24 hours from injection it had fallen to values lower than in the control group. These phenomena were explained on the ground that the high uptake of iodine in the thyroid of the mothers receiving a deficient diet resulted in a decreased diaplacental transfer of the nuclide available for uptake by the thyroid of the fetuses.

242. A third report [B7] is of relevance in the context of releases to the environment under normal or accidental conditions. It refers to man, and it contains calculations of the doses delivered to the thyroid glands of various age groups in the population (fetuses of 13-40 weeks of gestation, infants of 1 or 2 years, children of 5, 10, 15 years and adults), by different concentrations of inhaled or ingested iodine nuclides (^{129}I, ^{131}I, ^{132}I, ^{133}I). These calculations were carried out on the basis of assumed parameters concerning the weight of the thyroid, the biological half-life of iodine, and the amount of iodine ingested or inhaled. For the same atmospheric concentrations, the results indicate a greater fetal dose from inhalation, suggesting that the fetus is most susceptible to being affected by airborne releases of the relevant radionuclides. Based on data from animal studies, suggest-

ing that the thyroid sensitivity is 10-200 times higher in fetuses than in adults, the fetus was also found to be most susceptible to ingested short-lived radioiodine. As to the longest-lived ^{129}I, inhalation in adults and ingested milk in the young infants are the routes likely to deliver the greatest thyroidal doses.

243. The above data [B7] combine information on age-dependent dose per unit uptake, and per unit concentration in the environment (which includes age-dependent diets and breathing rates) and age-dependent sensitivity. This information is analysed separately in another model of iodine metabolism useful for calculation of the dose to the thyroid as a function of the fetal age in the fetus and mother described by Johnson [J11]. This model, based on available human data, indicates that the doses to the fetal thyroid from radioiodine taken in by the mother range from below the dose observed in the mother's thyroid to a factor of 3, at most, above it. The composition of the diet will determine whether ingestion or inhalation is the most important pathway. As to the sensitivity of the human fetal thyroid, Johnson and Myers [J12] have argued that there is no evidence to support large differences in the effects of ^{131}I and external irradiation in post-natal human thyroids, although differences by a factor of up to 10 cannot be ruled out. Doses to the fetal thyroid from chronic radio-iodine uptake by the mother over the gestation period would not be significantly different from those to the mother's thyroid. For acute exposure at any time during pregnancy, it may be calculated [J11] that the dose to the fetal thyroid is never more than a factor of 3 higher than that to the mother's. A recent careful review of the placental transfer and fetal binding of iodine in various mammalian species, including man, contains valuable information for inter-species comparisons [S85]. This is generally in line with conclusions in [J1] and [J12].

3. Phosphorus-32

244. In experiments by Krishna et al. [K17], pregnant mice received an intraperitoneal injection of ^{32}P (370 kBq) at 3.5 days p.c. and were then allowed to produce offspring. The incidence of successful copulations in this group of animals, compared with that in non-injected controls, was drastically reduced, indicating that there were losses of all litters prior to implantation. There was also a reduction of the litter size at birth and at weaning, due to post-implantation mortality during the early stages of development. The weight of the litters at birth was not apparently changed by the treatment, but subsequent growth was to some extent affected. These data are qualitatively in agreement with those that would be expected from external irradiation during the pre-implantation stages of development.

245. Radiophosphorus (37 kBq/g body weight) was also injected intramuscularly into pregnant Swiss albino mice on the 7th day after fertilization, but was not found to affect substantially the development of the pituitary gland, as judged by histological tests at 18 days p.c. or 14 days of extra-uterine age. Mice injected intraperitoneally with the same level of radiophosphorus when one day old, and autopsied at various times thereafter up to 42 days of age, showed some hypertrophy of acidophilic cells. In animals injected at 7 days, signs of cell death, followed by an increase of acidophilic cells, were found over the same time period. For injection at 14 and 21 days, a clear decrease of acidophils was found. These cells seem, therefore, the most severely affected by the injection of ^{32}P [D6].

4. Sulphur-35

246. Pregnant mice of the CBA strain were injected with 740 kBq of ^{35}S on 3.5 days p.c. The treatment had no effect on the mortality of the injected animals, but a slight non-significant decrease of the number of fertile matings was observed. Litter size was significantly decreased (6.4 young per female), by comparison with control animals not injected (7.5 young per female). A further loss of animals took place between birth and weaning, owing to a significant increase in the neo- and post-natal mortality of the offspring in the treated groups. The sex ratio and the whole-body weight of the young born to injected mothers were not altered with respect to non-radioactive controls [S3].

5. Selenium-75

247. Following a single administration of ^{75}Se-methionine to pregnant female rats at 11-13 days p.c., the state of the endocrine system of the progeny was evaluated at 9, 12, 18 and 26 months of age. To this effect the concentrations in blood of corticosterone, testosterone and estradiol were studied. Changes were seen to be more pronounced in the male animals [D20].

6. Fission products

248. Senekowitsch and Kriegel [S67] compared the placental transfer of fission products such as strontium, caesium, yttrium and cerium in the rat and found that the uptake by the fetus decreased from the first to the last nuclide. The mothers were injected intravenously with a standard amount of each nuclide 21 days before pregnancy, on the day of mating, and 10 or 17 days after conception. The activity concentration in the organs of the mother and the fetuses was determined on pregnancy day 19, at which time the fetal weight had a fairly large degree of variability, owing, presumably, to a very long mating period of 2 days. For each nuclide, the authors found a statistically significant positive correlation between the deposition in the fetus and its weight. Uptake also depended on the day of development and was higher the later in pregnancy administration took place. The concentration of ^{131}I in fetal organs at 8 hours post-injection showed a very large increase (by a factor of about 1000) between 17 and 20 days of intra-uterine age, particularly between days 17 and 18. Thyroid activity compared with whole-body activity was 0.44% on day 17 and it increased to 35% by day 19.

249. In work by Villiers et al. [V11], sows were chronically treated with ^{85}Sr by intraperitoneal injection twice weekly of 185 kBq, in order to follow uptake and retention in the pregnant mother and fetus. The state of pregnancy did not modify these parameters appreciably. At birth, piglets had an ^{85}Sr content amounting to about 0.5 to 1.0% of that of the delivering mother, and the radionuclide taken up in utero was only excreted very slowly. Piglets were fed after delivery by either contaminated or control sows to study uptake through the milk. Those fed by contaminated sows increased their radionuclide content by about three times before weaning, but excreted it quite rapidly thereafter, so that by about three weeks they contained the same amount of ^{85}Sr as at birth. Some aspects of the metabolism of ^{131}I in the ruminant, including pregnant and lactating animals, were also reported on by Daburon [D26].

7. Lead-203

250. At 8-18 days of gestation, pregnant mice of the C57BL strain received ^{203}Pb-nitrate intraperitoneally [D22]. The whole animals, or their excised uteri, were subjected to autoradiography or autopsied for scintillation counting of the excised organs. Lead was found in the embryonic and fetal tissues after injection at all gestational ages. On days 8-11, it appeared mainly in embryonic blood, suggesting that it was essentially incorporated into the haemoglobin of yolk-sac erythrocytes. Starting from day 12, lead was found in liver (possibly in connection with the haematopoietic function of this organ) and in the skeletal cartilage and from day 14 on in the ossified skeleton. Thus, the overall fetal concentration increased progressively with gestational age. No uptake was observed in fetal kidney, in spite of accumulation in proximal tubuli of the maternal kidney.

8. Thallium-204

251. The uptake and retention of thallium in mice during gestation was investigated by autoradiographic techniques with ^{204}Tl sulphate [09]. About 1.85 MBq of the nuclide were injected in the peritoneum of 15-day pregnant females. Fifteen minutes after injection, the nuclide could be traced in the fetuses. The maximum fetal accumulation was seen at 2-4 hours post-injection and the minimum at the last observation time, which was 4 days after injection. Observations at 5-15 days of gestation showed that thallium crosses the placental barrier at any time. It is retained by the visceral yolk-sac placenta during early pregnancy and also by the chorioallantoic placenta and the ammnion during late gestation.

9. Transuranic nuclides

252. Although a fair amount of new information on plutonium and other transuranic compounds administered to pregnant animals has become available, this refers more to the transplacental movement and distribution of the substances rather than to the effects induced on the product of conception. In the BALB/c mouse, Weiss and Walburg [W9, W21] measured the placental transport of plutonium citrate after intravenous injection of three different concentrations of ^{239}Pu during the late stages of pregnancy. The animals were killed 48 hours post-injection for tissue preparation and analysis. The authors noted that there was an increase in the percentage of plutonium incorporated into fetal tissue with decreasing activity down to levels of 3.7 kBq per animal. This was attributed to a mass effect. This pattern of relationship was also noted for the placental tissue and was explained by the fact that a body compartment, most likely the liver, retains a greater fraction of the radioactive material at higher levels.

253. Similar and parallel experiments [W10] were also performed with americium-243 administered intravenously as citrate in various concentrations (between about 0.4 and 130 kBq/mouse) to pregnant BALB/c animals 16 days after mating. These concentrations were chosen in such a way that the number of atoms in each injected dose would be equal to the number of ^{239}Pu atoms in the study reviewed above [W21]. Forty-eight hours after injection, the concentrations of the nuclide were determined in fetuses, placentas, in the maternal femur, liver, carcass and pelt. It was found that, atom for atom, americium was incorporated into fetal tissues 10-25 times less than plutonium at similar concentrations. Here again, however, at low dose levels the average fraction incorporated into the fetuses decreased with increasing activity administered to the pregnant mothers. The same could be said for the placenta and the femur. The mass effect for americium was about a factor of 2 within the concentrations covered, that is slightly lower than that of ^{239}Pu which was nearly a factor of 4 [W21]. A scavenging effect by the maternal tissues, and particularly by the liver, was discussed as a plausible explanation.

254. The point was made, regarding these two studies [W10, W21], that extrapolations from animal to man should always be made at the low levels of environmental human exposure, which would be hundreds of times less than for the experiments reviewed. It is doubtful whether activity analyses at such extremely low levels of contamination are at all possible. However, the concept should be borne in mind that perhaps current environmental levels of the nuclides might be more readily transferred from pregnant mothers to the fetus than for the usual much higher experimental level uptakes.

255. New-born rats from dams injected with 49 kBq ^{239}Pu citrate kg^{-1} body weight 38-53 days prior to parturition contained an average of 0.008% of the maternal injected dose per neo-nate [T16]. Also in the rat Hisamatsu and Takizawa [H29] studied the placental transfer and distribution of ^{241}Am by injecting the radionuclide under the form of citrate at 15 or 18 days of gestation. The animals injected at 15 days were killed at 2, 24, 48 or 120 hours and those injected at 18 days were killed 24 hours thereafter. An autoradiographic study of the nuclide distribution in the fetus, fetal membranes and placenta showed that

the deposition in the feto-placental units increased with gestational age. Major deposition sites were the skeleton and liver, but the presence of the nuclide in the yolk sac splanchnopleure and in the exocoelom suggested also an important role of the yolk sac placenta in the transfer mechanisms. The post-natal development of rats treated with ^{241}Am was studied by Ovcharenko [O13].

256. In the guinea-pig (outbred Harley strain), Kelman and Sikov [K5] measured the movement of ^{239}Pu in vivo at 59 to 61 days of pregnancy by perfusion of the fetal circulation, a technique which avoids the complications occurring through fetal accumulation of the nuclide. The perfusion technique also entails continuous monitoring of the pressure, heart rate, electrocardiogram, blood pressure and respiration rate of the mothers. The pregnant animals were administered trace amounts of tritiated water in order to monitor changes in the maternal blood flow to the placenta and about 1 MBq/kg of ^{239}Pu citrate intravenously. This dose of plutonium is of the order of the $LD_{50/30}$ for other species (about 740-3000 kBq/kg). Clearance of plutonium from the mother to fetus was found to be small, amounting, for example, to less than one-fifth of that for inorganic mercury. One factor that might account in part for such a low clearance is the placental blood flow of the mother, which is greatly depressed in the animals treated with plutonium.

257. In an abstract by Andrew et al. [A8], valuable data were provided on the distribution of ^{239}Pu in the gravid baboon. Following previous evidence that, in the rat the placenta and the fetal membranes attain substantially greater concentrations of the nuclide than does the fetus, and that these observations may not be easily extrapolated to man owing to large interspecies differences, the authors chose the baboon as an experimental animal. In this species, in fact, development of the placenta is similar to that in man. Pregnant animals at various stages of gestation were administered 370 kBq/kg of monomeric ^{239}Pu intravenously. The uteri and their content were then removed surgically 24 hours later for radioanalysis and autoradiography. The mothers themselves were allowed to live an additional two to six days. The fraction of dose reaching the feto-placental unit, and the ratios of the concentrations in the fetus to those in the placenta, were found to be very similar in the rat and the baboon, when similar stages of gestation were compared. If, in addition, the morphological differences in placentation were allowed for, the autoradiographic distribution of plutonium was found to be remarkably similar in the rat and the monkey.

258. Joshima et al. [J3] studied the effects on embryonic and fetal haematopoiesis of ^{239}Pu citrate administered intravenously to the Wistar rat (about 1.3 MBq/kg, no dose estimate available) on gestation day 9. At this time, the yolk sac begins to form the blood islands and also selectively concentrates monomeric plutonium. The injected animals were killed 5 or 10 days after injection to measure any effects in the circulating blood, yolk sac and fetal haematopoietic organs. The treatment increased pre-natal mortality and reduced maternal (but not fetal) weight gain. At both sampling times the pregnant mothers showed a decreased number of reticulocytes and leukocytes; the red blood cells were also lower at 10 days. The fetuses showed a transient decrease in the concentration of non-nucleated mature erythrocytes and changes in the distribution of cell types of the erythropoietic series, which were interpreted as disturbances in the maturation processes. The presence of plutonium also reduced the mean weight of the yolk sac and the fetal liver. In both these organs, and also in the spleen, the mean number of cells was decreased, without any shift in the distribution of the various cell classes.

259. Kelman, Sikov and Hackett reported [K6, K23] on the effects of monomeric ^{239}Pu on the fetal rabbit. Animals at gestation days 9, 15 and 27 received 370 kBq/kg body weight of monomeric ^{239}Pu intravenously and were killed one day later; no estimate of absorbed dose was provided. Groups were also similarly injected at 9 and 15 days and killed at the 28th day of gestation. One group, injected on day 9 and killed on day 28, received a four-fold higher activity concentration. Conceptuses were examined for viability at 10, 11 and 29 days. Each fetus from litters injected at 9, 15 and 28 days, and killed on day 29, was weighed, measured and examined for sex, external abnormalities and cleft palate. Exposure to about 1.5 MBq/kg decreased fetal weight significantly, compared with that of the group receiving 370 kBq/kg, while the smaller weight losses induced by this latter activity concentration were not thought to be significant. Foetal mortality was significantly increased, except in groups receiving the lowest activity on days 9 and 15. Only 5 out of 377 fetuses examined were abnormal. This low number, and the lack of any pattern in the induction of malformations, made any association between their incidence and the exposure to plutonium rather unlikely. Subtle alterations, which could not properly be classified as malformations, were detected in the skeleton of the exposed litters and there was a general retardation of the skeletal development in the group receiving the highest activity of plutonium.

260. Ovcharenko and Fomina [O6] reported on the effects of ^{237}Np oxalate administered intravenously to rats. They found an increased pre-implantation mortality in females. The progeny of these rats receiving 11-185 kBq/kg showed a decrease in the rate of production of red blood cells after gamma irradiation, together with a prolongation of the period of narcosis after administration of hexanol.

B. CONCLUSIONS

261. Although a number of new contributions regarding the effect of internal irradiation on the mammalian conceptus have appeared since the matter was last reviewed by UNSCEAR [U2], the information gathered is not systematic in regard to the nuclides tested, their chemical form, the route and schedule of administration, and the kinetics of transfer and metabolism of the substances from the mother to the feto-placental complex. Complete dose-effect relation-

ships are seldom reported. Also, data regarding various animal species, developmental stages at treatment, and type of effects scored, are very sporadic. As a consequence, any attempt to extrapolate between nuclides, doses, species, developmental stages or any other major physical or biological variable appears premature. From the long list of nuclides for which scattered information is available, three stand out as having received somewhat better attention: tritium, iodine-131 and plutonium-239. The fact that these also happen to be, potentially, among the most important for human exposure under normal or accidental conditions is probably the reason for the interest they have received. It does not mean, however, that the information available for them is very satisfactory.

262. For tritium, many contributions refer to irradiation during the pre-implantation stages in vitro. In the culture medium, activity concentrations of tritium oxide of the order of 4-16 kBq/ml have been shown to stop the progression of 50% of the zygotes to the blastocyst stage, the variability between these values being due in large part to the developmental stage of the early embryo at irradiation. Embryo sensitivity generally decreases with increased differentiation. Tritium in the form of thymidine is about 1000 times more effective in producing such effects than tritiated water. Tritium oxide and tritiated thymidine also act differently on various developmental stages. Tritium is metabolized by the embryo and its organs according to different kinetics: the loss of the radionuclide per gram of tissue in the new-born after uptake in utero shows a fast component with a half-life of a few days followed by slower components linked to the metabolism of the organically bound isotope. This has been established in both the mouse and the pig. The dose received by cells in each tissue is therefore governed by the level of tritium in the body fluids and by the specific cell kinetic and metabolic pattern of each tissue. Whole-body doses of the order of 1 Gy or less from tritium have been reported to produce changes in litter size, sex ratio, and neo-natal mortality in the mouse. They also cause increased tumour incidence and changes in the life span. Sustained levels of tritium in the drinking water of pregnant rats at concentrations of about 100 kBq/ml produced in the offspring some biochemical sign of cerebral damage (low DNA and protein content), although the weight of the brain was normal. Continuous administration of tritiated water over 5 generations showed no sign of cumulative effects. Other patterns of tritium dosage at similar concentrations also produced evidence of brain defects which could be followed well into post-natal life. There is evidence that organically bound tritium is more damaging than tritiated water for the same administered activity. The RBE for a variety of end-points (dominant lethal mutations, chromosomal aberrations, chromatid exchanges, alterations in bone marrow cells) following HTO ingestion in the mouse, compared with external gamma rays from ^{137}Cs, was assessed to be between 1 and 2.

263. Many of the experiments using radioactive iodine appear directed to clarify the relationship between the amount of activity administered to the dam and its route of uptake, on the one hand, and the level of iodine in the fetal tissues, particularly in the thyroid, on the other. The transplacental transfer of radio-iodine as a function of time from the uptake by the mother has been studied in rats and in the guinea-pig. So have the relative concentrations in the blood of the mother and of the fetus and in the amniotic fluid, and the relative uptake in the mother's and offspring's thyroid. They have also been estimated in man on the basis of some assumed parameters of uptake and metabolism. As a result of using simplifying calculations, it has been provisionally concluded from these data that for acute exposure at any time during pregnancy the dose to the thyroid of the fetus is never more than three times higher than that of the mother. For chronic uptake over the whole gestation period these doses would not significantly differ from each other.

264. Experiments on ^{239}Pu contamination of pregnant mice have shown that the proportion of this nuclide incorporated into the fetal tissues tends to be relatively higher (a factor of 4, as a maximum) with activity concentrations decreasing down to about 3.7 kBq per animal. Although atom for atom, ^{243}Am is incorporated 10-25 times less efficiently than plutonium at similar concentrations, the average fraction of administered activity incorporated into the fetus decreases also for this nuclide with increasing mass of the nuclide to the pregnant mother, by a factor of about 2. The presence of such effects should be borne in mind when assessing the uptake of environmental levels of transuranic nuclides, which are orders of magnitude lower than the experimentally tested levels.

265. Comparisons of the distribution of ^{239}Pu in the mother and fetus in the rat and the baboon have shown that when similar developmental stages are compared, and morphological differences in the placenta of the two species are allowed for, the distribution of the nuclide is very similar. While existing data are insufficient for precise estimates, no clear tendency of plutonium to concentrate into the fetus has been documented. There appears to be, on the contrary, some tendency of the placenta and fetal membranes to take up the plutonium that might otherwise be transferred to the fetus. Experiments on the effects of plutonium are relatively less common than those on its distribution. However, some haematological changes have been documented in the rat at about 1.3 MBq/kg of ^{239}Pu citrate administered on gestation day 9. Fetal weight loss and mortality (but not malformations) were significantly induced by concentrations of monomeric ^{239}Pu in the range of 1 MBq/kg administered to pregnant rabbits.

266. There appears to be an urgent need to enlarge the data base for the nuclides of most practical importance with respect to uptake, distribution in the feto-placental complex, and possible effects in the offspring, over a larger variety of radioactive chemicals and a wider range of nuclide concentrations. The importance of correlating effects to tissue doses rather than intake activities should also be emphasized. In addition to providing much needed information of potential value under conditions of normal operation and accidental exposure to radiation sources, such

new data might contribute to bringing about generalized conclusions on dose-response relationships for pre-natal internal exposure to radioactive nuclides.

VI. THE ROLE OF MODIFYING FACTORS

267. The present chapter deals with physical, chemical or biological factors that have been shown to modify the radiation response of pre-natally irradiated mammals. It focuses on radiation type and energy and the effects of combined actions, including radioprotective and radiosensitizing treatments.

A. RADIATION QUALITY

268. The insufficiency of information concerning the effect of radiation quality and dose rate, in the context of teratogenic damage and pre-natal irradiation effects in general, was pointed out by UNSCEAR in its previous review of the subject [U2]. It was concluded at the time that the data only allowed some speculation about RBE factors in respect of irradiation in utero. However, this knowledge was compatible with the notion that higher RBE factors applied to the more densely ionizing radiation and to low-LET dose protraction, as compared with high-dose-rate and low-LET radiation. UNSCEAR called specifically for a systematic exploration of this area, and over the past few years new information has in fact been produced. This information is reviewed below, according to the various types of radiation tested.

1. Neutrons

269. In experiments by Molls et al. [M33], preimplantation mouse embryos in the G_2 phase of the 2-cell stage were exposed in vitro to either x rays (240 kV) or fast neutrons (average energy, 7 MeV). At various times thereafter (up to 63 hours post-irradiation and growth in culture), these embryos were scored for the number of cells per embryo and the number of micronuclei per cell. Both radiations did induce the formation of micronuclei at very low doses, and the kinetics of induction was found to depend on radiation dose and the length of the division delay during the first and second cell cycle after irradiation. New micronuclei were appearing even after the third and later post-irradiation mitoses, in accordance with the notion that radiation-damaged cells do not all die at the first post-irradiation division. The number of micronuclei per cell at 39 hours post-irradiation appeared to follow an essentially linear increase with the dose of x rays and a convex upward response with the dose of neutrons. An x-ray dose of 0.06 Gy was sufficient to double the average number of micronuclei per cell of the control embryos; 0.03 Gy of neutrons produced a 6-fold increase over the control values.

270. Cairnie et al. [C3] reported on teratogenic and embryolethal responses in CF_1 mice irradiated in the Janus reactor at the Argonne National Laboratory in the United States. At this facility the mean energy of the neutron spectrum was approximately 0.8 MeV; thermal neutrons and gamma rays contributed less than 1% and less than 3%, respectively, of the absorbed dose within the mice. Neutron effects were compared with those of ^{137}Cs or ^{60}Co gamma rays. Irradiation took place at 3, 5, 8 and 11 days p.c. Doses were 1, 1.5, 2.0 and 3.0 Gy for gamma rays and 0.25, 0.5, 1.0, 1.5 and 2.0 Gy for neutrons. Offspring were examined on day 17 of pregnancy for the incidence of resorptions and dead or viable fetuses. Live fetuses were also scored for gross external abnormalities and body size. The same types of responses were observed for neutrons and gamma rays. For lethal effects and malformative effects considered as a single class, the RBE in terms of average absorbed dose to the embryo was estimated to be 2.9 (the corresponding ratio in terms of kerma in free air was 2.5). Taking the average number of abnormalities per fetus as the end-point, the findings also pointed to an RBE of between 2 and 3. The highest incidence of abnormalities was obtained for irradiation on day 11, when malformations of the limbs were especially frequent. Approximate values of RBE estimated for those abnormalities that were sufficiently numerous were in the range of 2 to 3, usually closer to 3. It was pointed out that these RBE values were consistent with other values for effects dependent on cell killing in vitro and in vivo which, for the particular radiation sources used, were between 2.2 and 4.

271. Although no comparison between different types of radiation was involved, Vogel [V3, V4] exposed female Sprague-Dawley rats on pregnancy day 18 to single whole-body doses (0.2 to 1.5 Gy) of fission neutrons of 1.2 MeV average energy (neutron/gamma dose ratio approximately 7, dose median LET approximately 50 keV/μm). A dose of 0.2 Gy caused a small decrease of body weight lasting from birth to weaning. During this period, 9% of irradiated rats died, compared with 4% of the controls. After 0.5 Gy, about 24% of the irradiated animals died within the same period, with a more profound body weight loss of the survivors. One Gy caused about half of the rats to die within 24 hours of birth and over three-quarters before weaning, with a large and significant loss of weight in the survivors. No survivor among 95 rats receiving 1.5 Gy was left within 2 days of birth. The neutron LD_{50} from birth to weaning was estimated to be 0.75 Gy. Organ weight changes were followed between birth and 1 week of age and they showed an overall decrease of the measured values in irradiated animals. The most significant weight loss was found in the CNS and in the testis one month after neutron exposure. These effects were thought to be directly induced in the irradiated fetuses and not secondarily through irradiation of the mother.

272. Di Majo et al. [D7] compared the effects of fission-spectrum neutrons (zero to 0.465 Gy, average energy ~0.4 MeV, gamma contamination ~12.5% of total dose) with those of x rays (250 kV, 0-2.0 Gy at 0.13 Gy/min). The end-points scored included embryo lethality, weight reduction and teratogenic effects (gross malformations and minor anomalies). The

animals used were F_1 hybrids of C57Bl and C3H mice, irradiated at 7.5 days p.c. They were scored for the above effects at 18.5 days p.c. Mortality curves found for both radiations were highly curvilinear, with a lower "threshold" for neutrons than for x rays. The ratio of x-ray to neutron doses (1.4 and 0.47 Gy, respectively) to produce 20% mortality was approximately 3. The loss of mean fetal weight was approximately linear with increasing dose of both radiations, and the ratio of the slope regressions gave an RBE of 3.2 ± 0.8, as an average over the dose range tested. Highly curvilinear relationships with dose were observed for major malformations after x-ray irradiation, with thresholds of the order of 1 Gy. Dose relationships for neutrons, however, showed no apparent thresholds, so that the RBE of neutrons was very dependent on neutron dose. The equal effectiveness dose ratio estimated at 7% incidence of severe external malformations was 2.4, increasing to about 3.4 if other, more common, head abnormalities were also included.

273. Preliminary studies of dose-response relationships for developmental damage in the CNS of the mouse after pre-natal exposure to x rays, fast neutrons and ^{131}I were also reported [K41]. RBE values following x-ray or neutron exposures were established in relation to a number of morphological parameters such as the thinning of cortical plate, corpus callosum and fimbria hippocampi. They were shown to vary between 3 and 4, according to different times and effects. The studies included analyses of neuronal branching defects by computerized micro-video techniques, as a function of dose. There was evidence that "thresholds" existed in the region of 0.1 Gy for such types of structural damage.

274. The effects of a single whole-body dose of 0.5 Gy of neutrons (peak energy about 3 MeV) on mortality and body and brain weight in $(DBA_2 \times C57BL/6)F_1$ hybrid mice were described by Antal and Vogel [A10, V9]. Radiation was delivered at 17 ± 2 days p.c. This treatment caused a significant increase of death within two weeks after birth, from a level of about 12% in the control animals to about 41% in irradiated ones. The average birth weight showed a 15% reduction in the irradiated mice. This weight loss was not recovered until 6 months after birth. Brain weight at 3 weeks of age was 30-35% less in the irradiated mice. Early mortality and brain weight loss were not seen in mice irradiated at 1-7 days from birth. The significance of these data, particularly in regard to timing, was discussed in relation with the induction of severe mental retardation of children exposed in utero to the atomic bomb explosions. The authors' opinion is that the time of most active neuroblast proliferation is, in both species, the most vulnerable period for radiation exposure.

2. Helium ions

275. In a series of papers, Ward and his group [W2-W6] reported extensively on the effects induced by irradiation in utero by a helium ion beam generated in a synchro-cyclotron. By suitable ridge filtration, doubly charged 530-MeV monoenergetic helium ions were modified into a heterogenous beam with an extended Bragg peak, delivering essentially a uniform radiation dose at a depth of 4-10 cm in water. This allowed animals to be exposed rather uniformly from a lateral direction. The LET spectrum was such that at 8 cm about 10% of the dose was due to tracks of LET higher than 20 keV/μm; and about 1% to tracks of LET higher than 100 keV/μm. Cobalt-60 gamma rays were used as the standard radiation for comparison.

276. In the first experimental series [W2, W4], Sprague-Dawley rats were exposed on gestation day 8 under aerobic or hypoxic (95% helium and 5% oxygen) conditions in the dose interval of 2-12 Gy. The uterine content was examined on gestation day 20 for live and dead fetuses, resorption sites, weight and gross morphological abnormalities. The number of corpora lutea served as an index of the number of ovulations, and embryonic survival was expressed as a per cent of live fetuses over the number of corpora lutea. The results may be summarized as follows:

Survival	*RBE*		*Oxygen enhancement ratio*		*Oxygen*
(%)	*Aerobic*	*Hypoxic*	*Helium ions*	*Gamma rays*	*effect gain*
50	1.0	1.2	1.7	2.2	1.2
10	1.1	1.4	1.5	2.0	1.3

Thus, the high-LET component of the helium ion beam had a negligible effect on the RBE under aerobic conditions, but lowered the effective oxygen enhancement ratio of the beam by about 25% relative to that of the gamma rays. Helium ions were significantly more effective than gamma rays in stunting fetal growth under hypoxic and aerobic conditions (1.5 and 1.7, respectively) and in producing gross morphological abnormalities under aerobic, but not under hypoxic, conditions. In fact, at 10% incidence of abnormal fetuses, the equal effect ratios were about 1.7 and 1 under oxygenated and hypoxic conditions, respectively.

277. In another paper [W3], the embryonic survival was examined in rats exposed to split doses of radiation delivered in two fractions at 5 and 6 days p.c. Helium ions, which showed a single-dose RBE (with respect to ^{60}Co gamma rays) of 1.0, had a split-dose RBE of 1.5, in accordance with expectation if the killing curves for helium ions had a smaller shoulder than that of gamma rays.

278. Finally [W5, W6], Sprague-Dawley rats were exposed to 0.5-6.0 Gy of ^{60}Co gamma rays, or to extended Bragg peak helium ions, on days 3-10 of pregnancy, and the uterine content was examined on pregnancy day 20 for scoring of resorptions and dead or live fetuses. The number of dead fetuses was referred to the number of corpora lutea to calculate the percentage of killing. The sex of the fetuses was also scored. For gamma irradiation, the LD_{50} in utero ranged from 1.4 Gy on day 3 to 5.15 Gy on day 6 of gestation. For helium ions, the values ranged from 1.7 on day 10 to 4.9 Gy on day 6. Day 6 (the time of implantation) was the most resistant stage for both radiations, while the early organogenesis period (days

9 and 10) was found to be the most sensitive. In spite of the variation of radiosensitivity over a factor of 3 to 4 during the period of development examined, the magnitude of the fluctuations at the various times was essentially the same for both radiations, so that the RBE of helium ions with respect to gamma rays was, on average, 1.0 during the whole period. A consistent feature in irradiated litters was the preponderance of male fetuses among the survivors, for which no explanation is available.

3. Negative π mesons

279. In experiments by Michel and collaborators [M4], mouse embryos in the pronuclear-zygote stage (gestation day zero) were irradiated with either 140-kV x rays or negative pions to a dose of 0.135 Gy. The animals were examined when they reached the fetal stages on gestation day 13. The developmental effects scored included intra-uterine death, retardation of growth and incidence of malformations. Significant decreases in the percentage of normal implantations were obtained with π mesons at the peak and with x rays. The effect of the pions at the plateau was less important. Irradiation with peak pions was found to be more effective than with x rays, by a factor of about 1.7.

B. COMBINED ACTIONS

1. Various chemical substances

280. Most of the data published since 1977 on the combined effects of radiation and other agents, taking as end-points various types of damage to embryonic systems, have been obtained by treatment of the very early stages of development and in vitro culture of the treated embryos. General aspects of the interaction between radiation and chemicals during pre-natal development were discussed in annex L of the 1982 UNSCEAR report [U5] and were reviewed by Streffer [S17, S36].

281. The same author and his group have used extensively the in-vitro culture of the early mouse embryo to test a number of substances in combined-treatment experiments. Following the order of publication, they reported on the interaction between ionizing radiation and lead [M31, V6], actinomycin D [M29, S19], phenols [M27], sodium nitrite [M16], cadmium [M28], ethidium bromide [M29], 2,4-dinitrophenol [M19], sodium sulfite [M43] and caffeine [M43].

282. The combined effects of these substances with x rays were studied, taking as end-points the development of 2-cell mouse embryos to hatching of blastocysts, the cell proliferation kinetics in these embryos, and the formation of micronuclei as an index of cytogenetic damage. The substances were usually added to the culture medium directly before x irradiation. Additive effects were found for all these end-points when x rays were combined with most chemical substances. This was the case for phenol, p-nitrophenol, 2,4-dinitrophenol, sodium nitrite, sodium sulfite, 2,4,6-trinitrobenzene sulfonate and ethidium bromide.

283. A supra-additive (synergistic) effect was observed with the combination of x rays and actinomycin D, cadmium chloride or lead chloride on one of the parameters. Only with very high concentrations of caffeine did a supra-additive effect occur on the three parameters. The supra-additive effects were generally seen under conditions when recovery processes after irradiation were inhibited. Of special importance is the finding that comparatively low concentrations of lead chloride [M31] and of actinomycin D [S17, S36] induced such potentiation of the radiation effects.

284. In the field of combined actions, a few other scattered observations have also been reported. Sikov and Mahlum [S12] found that the intravenous administration of 5.5 kBq of ^{239}Pu, which by itself was ineffective, to pregnant rats at either 8.5 or 9.0 days p.c. increased synergistically the teratologic and embryocidal effects of trypan blue injected intraperitoneally (70 mg/kg) at these times of gestation. Trypan blue is itself a teratogen in the rat, at least up to 11 days p.c. [B29].

285. Yuguchi [Y2] treated pregnant ddN mice at 7 days p.c. with x rays (100 or 150 R) with or without ultrasound at 1.5 or 3.0 W/cm^2 (1 MHZ) for 5 minutes. The gross appearance of the offspring and the histology of their eyes were examined on the 18th day of gestation. The author found an enhancement of the embryolethal effects of 150 R by the highest dosage of ultrasound and an enhancement of the teratogenic effects of 100 R, also by the highest dosage. The histopathogenesis of the ocular abnormalities was carefully described in these experiments. The sensitivity periods in fetal mouse retina by the combined action of the cytostatic drug 5-azacytidine and x rays were investigated by Schmahl and Török [S6].

286. Sadovskaya et al. [S76] and Rantseva [R22] investigated the reproductive function and physical development of the progeny of non-inbred irradiated white mice. Females were exposed every other day to fractionated long-wave x radiation (10.2 keV) for 31 and 82 fractions (five of which were administered after mating with non-irradiated males) at doses of 0.13 or 0.26 Gy per fraction. From the number of offspring, the coefficient of reproduction and the body weight of the new-born, these treatments were apparently ineffective. However, if under the same conditions of irradiation an SHF treatment (ppm 5 MW cm^{-2}, 20 minutes per session) was also jointly administered, there was a tendency towards a reduction in fertility and a decrease in body weight, as compared with the effects of the two treatments given alone [L8].

287. Kusama and Yoshizawa [K37] reported briefly on intra-uterine death, malformations, body weight and sex ratios of mice exposed to 1 or 2 Gy of gamma radiation and 1 or 2 mg cortisone (administered in this order) on the 7th day of embryonic age. A dose of 1 Gy of x rays given alone produced no higher

mortality than that in the control, but a higher frequency of exencephaly, while both end-points were found to be higher after 2 Gy. Cortisone alone did not significantly alter any of the end-points scored. On the contrary, the combined-action groups showed rates of mortality and malformation which were twice as high as those induced by the same dose of radiation given alone, an observation implying a synergistic interaction.

288. In another series of experiments [K38], the same authors exposed pregnant ICR mice on their 7th day of gestation to various doses of gamma radiation (0.5, 1.0 and 2.0 Gy) and of caffeine (0.1, and 0.25 mg/g body weight). The drug in the combined-action groups was administered immediately after the appropriate radiation dose. Controls with no treatment, and with radiation or caffeine alone at the same doses, were also included. The end-points scored on the fetuses after killing of the pregnant mothers at 18 days of gestation were the same as in the previous series [K37]. Intra-uterine mortality increased as a function of dose following a highly curvilinear shape (upper concavity). There was no effect when caffeine was given alone, but the combination of radiation and caffeine resulted in a synergistic effect on mortality. A synergistic effect was also found in respect of the incidence of malformations both minor (polydactylia, micromelia, short tail) and serious (hydrocephaly, exencephaly, parietal hernia, cleft palate and lip). For induction of gross malformations, the dose-effect relationships for radiation alone or in combination were highly curvilinear. Body weight reduction was thought to be a good indicator of the growth-retarding effect of the treatments, but showed no signs of synergistic interaction. Finally, the sex ratio of live fetuses in the different treatment groups was not significantly different from control.

289. After showing that pre-conception exposure of ICR mice increased the incidence of anomalies and of tumours (mainly lung adenomas) in the offspring of treated mice [N4], Nomura [N13] tested tumorigenesis by irradiation in utero, followed by chemical treatment with urethane. Pregnant mice of this same strain were exposed to x rays (a single dose of 0.36 Gy) every second day between zero and 16 days of gestation. Neo-natal mice were also similarly irradiated within 1 day of birth and, in addition, other pregnant females received 1.59 Gy on gestation day zero. When 21 days old, the mice received subcutaneously 5 μM of urethane per gram body weight and were killed 5 months afterwards for scoring of tumours and malformations. Tumours were mostly papillary lung adenomas. Survival of the animals was decreased with irradiation on day zero, and body weight decreased with irradiation on days 4-10, and particularly on day 12 of gestation. Irradiation in utero alone did not change the natural tumour incidence. Irradiation on days 0-4 and 8, followed by administration of urethane, increased the number of tumours per animal, compared with both irradiation alone or urethane alone. It was speculated that unidentified events, induced by irradiation in the embryos and persisting through a large number of cell generations, may render the cells more susceptible to the post-natal treatment with urethane. Such events may be similar to those recently described in cells transformed in vitro by x rays and discussed in annex B to the present report.

290. In a short letter, Kinner-Wilson et al. [K42] reported on 2707 children who died in 1972-1977 of various malignancies, including leukaemia, and had been exposed in utero to various types of drugs and to x rays, separately or jointly. These children were matched to 2298 control non-exposed cases. The increase in relative risks for "*all* drugs" and for radiation given jointly were significantly ($P < 0.001$) increased above the level of action of the agents given alone. When the drugs were divided into separate groups, only for two of the three groups (namely "sedatives" and "other unspecified drugs"), but not for the third ("hormones"), was there a significant association. In the view of the authors, these results cannot be attributed to artifact. The association could, in principle, be explained on one (or more) of the following mechanisms: an effect of the illnesses requiring the drug treatment; a true causal effect of the treatment(s); or an association between the drugs and another non-identified risk factor. Results are too preliminary to allow a choice among the mechanisms proposed.

2. Radioprotective and radiosensitizing treatments

291. Although part of the larger subject of radiation-drug interaction, the effects of radioprotective and radiosensitizing substances are traditionally treated separately. A number of reports have appeared on these subjects since the last UNSCEAR review [U2]. Radioprotective effects on embryonic systems were mainly studied by a group of research workers in India; radiosensitizing substances were mainly investigated in Switzerland.

(a) Radioprotective treatments

292. The drug 2-mercaptopropionyl glycine (MPG), a $-SH$ radioprotector, was used in the studies by Dev and her group. A variety of end-points were used to assess the protective action, including organ and whole-body weight changes and functional tissue effects. Pregnant Swiss albino mice were exposed to gamma rays (250 R) at 14.25, 16.25 and 18.25 days of gestation. The post-partum weight of the offspring was remarkably improved by pre-irradiation treatment of the mothers with 20 mg/kg body weight of MPG. Of the three ages tested, the earliest was the most sensitive [D4]. Similar observations were made on mice receiving 150 R [D19] and 50 R [D5] at the same times p.c.

293. In another series [G9], pregnant Swiss albino mice at 11.5 days p.c. were injected intraperitoneally with a single dose of about 5.6 MBq of ^{131}I per mouse. Other mice received MPG (20 mg/kg) 30 minutes before the ^{131}I and daily on each subsequent gestation day until parturition. Finally, a third group of mice was similarly treated with distilled water. The body

weight of the litters born to these animals was checked, starting on the day of birth and for 6 weeks thereafter. Organ weights of the animals at 6 weeks were also obtained. MPG afforded a modest degree of protection against loss of total body weight, and marginal protection in regard to the weight of brain, kidney, testis, spleen, thymus and liver.

294. As to tissue effects, 50 R of gamma radiation from ^{60}Co, given at days 14.25, 16.25 or 18.25 resulted in a depression of the leukocyte count of the young 1 or 2 weeks after birth, with recovery by the 4th week. Treatment of the mothers with MPG (20 mg/kg) 15-30 minutes before irradiation significantly reduced the leukocyte depression at 1 or 2 weeks of age [G7]. In other experiments [G13] mice were irradiated in utero at 14.5, 16.5 and 18.5 days p.c., with or without pre-treatment of the mothers with 20 mg/kg body weight of MPG. Doses of ^{60}Co gamma rays were 0.5, 1.5 and 2.5 Gy. Male animals were killed when 6 weeks old and the weight of their testes or the total number of spermatids in tubule sections were assessed. The radiosensitivity of the testes was found to increase with increasing fetal age. There was also some protection by MPG of the testes according to both end-points scored, but the amount of protection was variable and not evaluated over a large range of doses.

295. Palyga et al. [P13] irradiated embryonic (1-14 days p.c.) and fetal (15-23 days p.c.) rats while the pregnant mothers breathed a hypoxic gas mixture containing 10% oxygen and 90% nitrogen. A single dose of 2.0 Gy was used. Judging by the incidence of still-born, and by the per cent survival 30 days after birth, the treatment afforded a reliable degree of protection against lethality.

296. Turakulov et al. [T9] studied, in pregnant dogs, rabbits and rats the specific activity and biological effects of potassium iodide in various combinations with potassium perchlorate, as a prophylactic treatment for the protection of the thyroid gland against the effects of radioactive iodine during pregnancy. They reported that a combination of the two drugs in doses of 0.5 and 300 mg/kg, administered at any time during pregnancy, is biologically harmless and shows a high protective effect.

(b) Radiosensitizing treatments

297. The hypoxic cell sensitizer misonidazole was used by Michel and collaborators in interaction studies with low- and high-LET radiation. In one report [M5, M6], pregnant NMRI mice on the 8th day p.c. received intraperitoneally various doses of the drug (from 200 to 1000 mg/kg body weight). Single doses of 0.01 Gy of 140-kV x rays and 15-MeV electrons were given. The end-points investigated were embryonic and fetal death, malformations, and growth retardation as scored at 13 or 18 days p.c. Growth retardation was assumed to be present when the fetal weight was more than two standard deviations below the average control weight. Treatment with the drug alone at 670 or 1000 mg/kg was toxic to 13-day-p.c. embryos, while doses of 200 mg/kg were only marginally effective. On 18-day-old fetuses the drug alone (670 mg/kg) significantly increased the number of malformed living fetuses. X irradiation produced an insignificant decrease in the frequency of normal fetuses, while electron irradiation produced a marked increase of resorptions. The first observation is not surprising in view of the low doses given, but is apparently in contrast with the latter. As to combined actions, they were thought to be "at least additive" in the case of electrons and "synergistic" for the x rays. But whether the same observations might hold at other doses and times remains to be confirmed. Similarly, the differential effect of misonidazole in regard to x rays or electrons remains to be explained.

298. Michel et al. [M35] also reported on the combined effects of misonidazole with negative pions (0.125-1 Gy) or 200-kV x rays (0.125-1.5 Gy). Mouse embryos on the 8th gestation day were irradiated, with or without application of the drug, 30 minutes before exposure. The fetuses were examined on day 13 for lethality, growth disturbances or the presence of malformations. No significant killing effect was seen for either radiations in the dose range up to 1 Gy; however, the drug alone or in combination led to some lethality. Growth retardation was significantly present at 1 Gy and in combined treatments at lower doses. Multiple severe malformations and severe retardation were seen when a dose of 0.5 Gy was combined with misonidazole. The frequency of teratogenic effects (all included) was significantly higher at all doses for both the x-ray- and negative-pion-treated animals when irradiation took place after the administration of the radiosensitizer. There was, overall, a synergistic enhancement of radiation effects by the combined treatment, the extent of which varied as a function of the radiation dose and quality and also according to the induced effect.

299. In other experiments [M14], pregnant mice of strains F/A and NMRI were exposed to 0.01 Gy of whole-body pion- or x-irradiation at day 8 of gestation. Lucanthone (Miracil D), a known radiosensitizer in various biological systems, was administered intraperitoneally 30 minutes before irradiation at 35 or 70 mg/kg. Five days after treatment, the fetuses were observed for developmental anomalies (growth retardation, micro-ophthalmia, exencephaly). In both strains of mice, it was found that radiation produced a significant increase in the incidence of abnormal fetuses compared with non-irradiated fetuses. The effectiveness of negative pions (peak irradiation) was higher than for x rays with respect to the production of teratogenic effects, the RBE being between 1.7 and 1.9. The application of Lucanthone increased the number of damaged fetuses and led to various degrees of sensitization, depending on the mouse strain and dosage used. Differences between strains in respect of the incidence of malformations were explained by the possible slight difference of developmental stages at the time of treatment. A similar explanation was given for the strain differences found in the same mouse strains irradiated with 0.135 Gy in association with Lucanthone [M50].

300. Preliminary data were also reported by Balla et al. [B39] on the interaction between x rays (0.01 Gy) and the cytotoxic drug vindesine (injected intraperitoneally at 0.35 mg/kg 1 hour prior to irradiation) in mice on the 9th day of gestation. The end-points scored at 4, 12 and 24 hours after irradiation were mitotic activity and cell death in the neuroblasts of the optic vesicle. Results at 4 hours showed potentiation of the x-ray effects by a factor of 1.8, based on cell necrosis.

C. CONCLUSIONS

301. Knowledge of the effect of radiation type and energy in modifying the response of pre-natally irradiated animals is still insufficient, particularly in comparison with similar information related to other non-stochastic effects. However, for irradiation with fast neutrons of various energies, data are now available on the induction of cell killing and production of micronuclei in the early embryos; for embryolethal and teratogenic effects in mice; and for lethal effects and body or brain weight loss in rats. By comparison with the effects of x or gamma rays, in no case did neutrons show an RBE higher than 10 at the doses used, down to about a tenth of a gray of neutrons, irrespective of the neutron energy, dose range, developmental stage at irradiation and time at scoring of the effects. In most cases reported, the RBE values were actually lower than 5, although the values may vary to some extent for different types of lethal or developmental effects or malformations. Peaks of maximum sensitivity related to the stage of development of the various anatomical structures occur at the same time for neutrons as for low-LET radiation, but quantitative information is inadequate to state whether the amplitude of the sensitivity oscillations is consistently higher or lower than for low-LET radiation. Moreover, experience is too limited to recognize definite changes of the RBE as a function of dose, although somewhat higher values could be expected at low doses, judging from the form of the dose-induction relationships of the radiations under comparison in some of the experiments.

302. Information on the shape of dose-effect relationships for various end-points and on possible changes of this shape with radiation quality is, however, insufficient. This makes it difficult to predict whether, at doses of the order of 1 mGy or lower, the values of the RBE might increase appreciably, as is the case for many other non-stochastic end-points. There is still no information on the joint effect of LET and dose rate, dose fractionation, and state of oxygenation of the fetal or embryonic tissues, that would make it possible to extend to effects in utero generalizations known to be valid for other effects at the cell or tissue level in adult animals. For example, a lower sparing effect of fractionation with high-LET radiation, as compared with that of low-LET, has not been documented for systems developing in utero. Similarly, data are insufficient to say whether the RBE of different radiations is higher under anoxic than under well oxygenated conditions for a number of end-points in utero. At the same time, however, there is no indication that the developing conceptus may behave, in respect of these major radiobiological variables, in a manner that is grossly at variance with that to be predicted on the basis of adult mammalian systems.

303. A series of papers has reported rather extensively on the effects induced by pre-natal irradiation with 530-MeV monoenergetic helium ions. This radiation was slightly, but consistently, more effective than gamma rays in stunting fetal growth under hypoxic or well oxygenated conditions and in producing gross morphological anomalies under aerobic exposure. Dose splitting over 24 hours (for the same total dose administered) resulted in higher RBE values. The radiosensitivity of the embryo and fetus to killing in utero over days 3-10 of pregnancy showed oscillations within a factor of three to four but on average the RBE of helium ions with respect to gamma rays over the whole period considered was 1. Thus, there were no major features to be gained from these experiments in respect to sensitivity periods, effects of oxygen, or effects of fractionation that would not be in accordance with expectations from basic radiobiological knowledge of gamma ray and helium ion dose-effect relationships for cellular effects. An RBE value of 1.7 for peak π mesons was reported for irradiation of pronuclear-zygote mouse embryos scored at day 13 of pregnancy for a variety of developmental effects.

304. The technique of in vitro irradiation and culture of early mouse embryos has been used extensively in recent years to test for combined actions with a variety of substances added to the culture medium. In general, this technique produces information that is more valuable for general toxicological than for radiobiological purposes. However, additive effects were reported for the majority of substances tested, including phenol, p-nitrophenol, 2,4-dinitrophenol, sodium nitrite and sulfite, 2,4,6-trinitrobenzene sulfonate and ethidium bromide. In other cases, for some combinations of radiation-drug dosage or for some of the end-points scored, synergistic effects were described. This applied to drugs such as actinomycin D, cadmium and lead chloride, and caffeine. The wider significance of the findings in respect of the very low environmental levels of some of these substances, and of later stages of development, remains to be fully clarified. For exposures during the stage of major organogenesis, synergistic effects of radiation administered in conjunction with trypan blue, cortisone, caffeine, urethane, ultrasound and high frequency radiation were also reported in single instances. More systematic studies would be needed to substantiate these observations for a wider range of doses and effects.

305. Clear evidence has been gathered of a radioprotective action for the drug 2-mercaptopropionyl glycine for a number of effects at the tissue or whole-body level in pre-natally irradiated mice. On the other hand, misonidazole, a hypoxic cell sensitizer, has been shown to potentiate the action of radiation when administered in combination with low- and high-LET radiation and for a variety of teratological and developmental end-points. The same was true of another known radiosensitizer, miracil-D, which showed

different degrees of potentiation, depending on the strain of mice, the dosage used and the type of malformation scored.

VII. TUMOUR INDUCTION

306. This chapter contains separate reviews of information on animals and on man. It will not attempt to discuss the general problem of mechanisms of tumour induction by radiation, which has been considered by UNSCEAR on many occasions and most recently in annex B to the present report. It is not known at present whether the same mechanisms underlying tumour induction in adult animals apply to mammals irradiated in utero: knowledge of these mechanisms is not sufficiently advanced to allow separate analysis of carcinogenesis in adult and developing animals. The reader is therefore referred to annex B for an extended treatment of tumour induction mechanisms which, at least in relation to basic radiobiological aspects, are also assumed for the time being to apply to tumours induced by irradiation in utero. The subject of malignancy induction following pre-conception exposure is discussed in annex A to the present report (chapter VI, section A).

A. DATA FROM ANIMALS

307. Data on the relative susceptibility to tumour induction of animals irradiated before or after birth, which had appeared up to 1977, were already reviewed by UNSCEAR in annex I of its 1977 report [U2]. While noting the relative scarcity of such data, UNSCEAR had concluded that pre-natal irradiation of animals seemed to affect the growth and differentiation of tissues rather than their malignant transformation. The types of tumours induced by radiation in adult animals vary considerably with species and strains, and this was shown to be true for irradiation in utero as well. Up to the time of the 1977 review, there was no evidence, however, that pre-natal irradiation of experimental animals in utero could be more carcinogenic than irradiation of young or adult animals. The following text updates that review with recently published data. It also examines other reports not included in the previous analysis.

308. Carcinogenesis following pre-natal exposure, particularly in experimental animal systems, was reviewed by Sikov [S46]. In his paper, he emphasized the methodological difficulties of working on pre-natal animals, and discussed the likelihood of an increase in tumour incidence following exposure in utero being statistically significant at low doses with the group size normally used for such studies. He also drew attention to the need to consider patterns of effects and modifications of tumour spectra to avoid missing important effects. On the other hand, his paper illustrated the difficulties of increasing the doses to produce unequivocal evidence of effects, because irradiation of pre-natal stages may produce morphological and physiological effects in several tissues at doses that would not be considered high when working with adult animals. As a consequence, there is a narrow range of doses to show radiation carcinogenesis in pre-natal animals, which is a limiting condition for obtaining precise dose-response relationships.

309. Valuable methods of studying radiation oncogenesis in cell culture have been developed in recent years and are increasingly being used. Some of them rely on the use of embryonic cells exposed in utero and then tested for transformation in vitro. Such methods would allow, in principle, comparisons of susceptibility between cells of various fetal ages, of carcinogenic responses in vitro and in vivo [B18, B19, B20], and of interaction studies between radiation and various inducing and promoting agents. All this could be done taking as an end-point an effect such as cell transformation, which is thought to be closely related to in vivo carcinogenesis. There are, at the moment, some unsettled methodological points and difficulties in understanding the precise relationships between transformation in vitro and carcinogenesis in vivo. When these difficulties are resolved, cell transformation may emerge as a very useful tool to study the mechanisms of carcinogenesis in pre-natal stages. At the moment, however, it appears from an extensive review of the field [B31] that no significant contributions are being published along the lines suggested above.

1. The mouse

310. Rugh, Duhamel and Skaredoff [R18] working on small groups of CF1 mice exposed to x rays (100 R) at each day between fertilization and 18 days p.c. found that the tumour incidence in the offspring was not statistically increased by pre-natal irradiation, although it was higher among the females, whether irradiated or not. From these experiments, the evidence for any increase in tumorigenesis was on the whole equivocal, and it appeared that the long-term sequelae of embryonic and fetal irradiation related more to growth and total body weight (permanent stunting) rather than to tumour induction.

311. Agarwal et al. [A1] reported on the radiation dosimetry of an experiment in which a range of doses (0.1 to 2 Gy) was given at 10 or 18 days p.c. to mice in utero (strain not specified). Their paper is almost entirely confined to the physical aspects of the experiments and refers only to an increased incidence of lymphoma and breast and lung carcinoma.

312. In the experiments of Friedberg et al. [F4] pronuclear zygotes of mouse strain CD-1, randomly bred, were irradiated in utero by fast neutrons (0.15 Gy). Animals that survived up to 30 days of extra-uterine life were then kept and observed until their natural death, recording the cumulative mortality distribution, the percentage incidence of the principal neoplastic diseases and the mean age at death for each tumour class. The differences found between irradiated and sham-irradiated animals of the same sex were not found to be statistically significant.

313. Sasaki et al. have published a series of studies on carcinogenic effects induced in mice by irradiation at various intra-uterine stages. In the first of these studies [S1], (C57BL/6 × WHT)F_1 mice were exposed (200 R) at 12 or at 16-18 days p.c. and examined throughout their extra-uterine life for the induction of degenerative diseases, growth retardation and the presence of tumours. For irradiation at the middle intra-uterine stages, the prevailing effects to be observed were growth retardation, several congenital malformations and amyloid degeneration. Tumours were not in excess over non-irradiated controls in this group of mice, but there was a significant decrease of incidence of lymphoreticular, lung and uterine tumours. Lung, pituitary gland and ovarian tumours were significantly enhanced after irradiation at the late fetal stages; liver and skin tumours were only slightly increased, thymic lymphoma was not.

314. In another study [S2], data were published on mice of the same strain irradiated at 17 days p.c. (150 or 300 R), within 24 hours of birth (400 R) or at 5 weeks of age (400 R). Overall, the susceptibility to tumour induction increased with age, in the order from pre-natal to neo-natal to young-adult mice; however, the most remarkable changes were found in the spectrum of tumours produced. An excess of pituitary, pulmonary and hepatocellular tumours developed in mice irradiated in utero, but lymphoreticular neoplasms and myeloid leukaemia were not induced. For irradiation in the neo-natal stage, thymic lymphoma, pituitary, ovarian, harderian gland, but particularly hepatocellular tumours were found in excess. Exposure at the juvenile age resulted instead in a higher incidence of ovarian and harderian gland neoplasms. The complex of these findings points to the conclusion that the age-dependence of susceptibility to radiation carcinogenesis differs for different organs and tissues. The high susceptibility of peri-natal mice to x-ray-induced hepatocellular carcinogenesis found in this series was also specifically discussed in another paper [S71] and was deemed to be related to the high frequency of liver cell mitosis at the time of, or after, x irradiation.

315. Two studies by the same group are so far only available in an abstract. In the first one [S28], groups of B6WF_1 mice were exposed to 200-kVp x rays (150, 300 or 600 R) at 17 days p.c. The incidence of lymphocytic neoplasms was compared with that shown by mice irradiated at 1, 7, 35 and 195 days of age. Exposure in the late fetal stages produced significantly higher rates of such tumours, reaching about 23% after 600 R. Susceptibility to the tumours was lower in the fetal than in neo-natal and suckling mice, but not lower than in the young adult mice. Data on the breakdown of various classes of lymphocytic tumours were also reported. In the second study [S27] B6C3F_1, animals were exposed to x rays at 17 days p.c. and at days 1, 7, 35 and 105 (300 R). Animals were killed when 800 days old for study of kidney and cerebellar damage and of tumour incidence. The carcinogenic response at the fetal stage was far lower than that of neo-natal mice and the tumour spectra were very similar. In early post-natal life lymphocytic, liver and pituitary tumours developed with high incidences, while myeloid leukaemia and harderian gland tumours were most elevated when irradiation took place at 35 and 105 days of age.

316. The results of other experiments by the same group are still in press [S72]. These experiments were carried out on B6C3F1 mice irradiated at 17 days p.c., within 24 hours of birth and at 15 weeks of age. Doses of 1.9, 3.8 and 5.7 Gy of ^{137}Cs gamma rays were used. Gross and histological pathology were performed on the animals at death, but data were not corrected for competing death causes. Overall, tumour incidence in irradiated animals was not significantly higher than in controls, which had themselves about 90% tumour incidence. However, late fetal animals were found to be particularly prone to pituitary tumours, which were statistically higher than in controls and had the longest latent period of all induced neoplasms. After 1.9 and 3.8 Gy, given at 17 days p.c., the incidence of tumours of the lung was significantly higher than in controls. Malignant thymic and non-thymic lymphoma was also in excess and appeared after shorter latent periods in fetal mice given 5.7 Gy; however, the susceptibility was higher in early post-natal than in pre-natal mice. The latter animals also showed a higher incidence of liver tumours than the controls, but not higher than that of neo-natal animals. Overall, the tumour susceptibility of mice irradiated in the late fetal and in the neo-natal periods was similar, but young adults were little, if at all, susceptible to the induction of pituitary, lung and liver neoplasms. The young adults were, however, more susceptible to myeloid leukaemia and to tumours of the harderian gland.

317. In order to investigate the question of the high susceptibility to radiation-induced tumours in pre-natal animals, Schmahl and collaborators [S32] set up experiments with a 2-stage carcinogenesis model. The irradiation of the NMRI mice in utero (3 daily doses of 0.8 Gy on pregnancy days 11-13) was regarded as a possible initiating stimulus, the promoting treatment being represented by the skin application of the phorbol ester TPA (12-O-tetradecanoylphorbol-13-acetate). Mice were treated with the promoter between 12 and 26 weeks of their post-natal life and observed for another 70 weeks for development of skin neoplasms. In spite of some differential effects in regard to distinct diseases, the overall conclusion was that the TPA treatment did not result in a higher tumour yield (either of the skin or of internal organs such as marrow, lung, liver, ovary) than that observed after pre-natal x irradiation alone. This result, which is at variance with other observations in adult rodent skin, was attributed to the dysplastic nature of the skin after pre-natal irradiation, which fails to respond to TPA as does normal fetal and newborn mouse skin. A decrease in the synthesis of prostaglandin induced by pre-natal irradiation would be responsible for the altered susceptibility to TPA.

318. In another series of experiments by the same group [S33] pregnant NMRI mice were subjected to fractionated x irradiation during late organogenesis (11-13 days of gestation), during the early fetal period (14-16 days) or during both periods (11-16 days).

Various fractionation schemes, with total doses ranging from 2.4 to 7.2 Gy, were tested and the offspring were followed for 39 months for the presence of ovarian tumours. With daily doses of 3×1.2 Gy, applied either in late organogenesis or in the early fetal period, a significant increase in the number of tumours was observed, mostly tubular adenomas, with about 20% between luteomas and granulosa cell types. Lower doses appeared ineffective. Autoradiographic observations on fetal ovaries 1 or 6 days after irradiation during late organogenesis showed a depression of the proliferation rate which was thought to be correlated with the relatively low susceptibility to tumour induction during this period. There was a sharp increase in the frequency of ovarian tumours following daily doses of 6×0.8 Gy or 6×1.2 Gy on days 11-13. Ovarian cysts were also found and they reached the highest incidences in animals treated with doses of 3×1.0 Gy or 3×1.2 Gy on days 11-13. The lowest cyst frequencies were seen in animals receiving doses for 6 consecutive days, which had the highest incidence of ovarian tumours.

319. Kusama and Yoshizawa [K24] reported on a comparison of the carcinogenic action of ^{137}Cs gamma rays on male and female C57BL/6J mice between the fetal (15 day p.c., 1 Gy), or post-natal age (30 days of age, 1 or 4 Gy). Autopsies were performed at death; tumour incidence rate, average latent period and composition of the tumour spectra were the biological end-points scored. There was no difference in the gross tumour incidence between control and irradiated mice, whether irradiation took place before or after birth. Different methods for adjustment of the data for competing risks were applied and it was concluded that the Kaplan-Meyer method was appropriate for the purpose. Under such conditions of analysis, the incidences of thymic lymphoma and breast tumours in all irradiated groups (fetal or post-natal) were found to be similar to those of the control mice. Reticular tissue neoplasms, on the other hand, showed a consistently decreased incidence in all irradiated groups, with little evidence of any special trend of the pre- as opposed to post-natal mice.

320. Pre-natal x irradiation of NMRI mice on the 12th day of gestation was reported by Schmahl to induce both dysplastic and neoplastic disorders [S65]. Dysplastic changes (see also [S62] and [S63]) include hypotrichosis, alopecia and skin ulcerations, appearing at 2-4 months of age in about one-quarter of the mice receiving 0.5-2.0 Gy. As to neoplastic changes, their incidence in the irradiated mice appeared to be generally lower than in controls, but the various classes of tumours showed a different behaviour. For example, the incidence of leukaemia (after correction for competing causes) was decreased at all doses below the normal incidence of the non-irradiated mice. By contrast, lung tumours (both benign and malignant), ovarian (see also [S33]) and liver tumours were increased, with a peak incidence at 1 Gy. Under the condition of the experiments, this dose was shown to be the most effective for tumour induction. The liver appeared to be the most sensitive organ, both for irradiation alone and in experiments using the tumour-promoting agent phorbol ester TPA.

321. Covelli et al. [C9] reported on experiments designed to investigate the role of age and LET on the susceptibility to radiation carcinogenesis and life shortening. Graded single doses of x rays (250 kV, 0.5-7.0 Gy) or attenuated fission neutrons (average energy, 0.4 Mev, 0.09-2.14 Gy) were administered to BC3F1 mice of both sexes in utero at 17 days p.c., or to male animals aged 3 or 19 months. Irradiation of 3-month-old animals caused a measurable dose-related degree of life shortening associated with a higher incidence and rate of appearance of radiation-induced tumours. Irradiation during the pre-natal ages or at 19 months, did not produce significant shortening of life after both x-ray and neutron irradiation: there was, however, a significantly higher incidence of solid tumours and reticulum cell sarcoma. The high variability of control values in the incidence of neoplastic diseases, particularly of the reticular cell tumour, and the differential carcinogenic action of x rays and neutrons in animals irradiated in utero, do not allow precise conclusions about the overall comparative susceptibility of mice irradiated at different ages. It is quite evident, however, that the changes in the tumour spectrum are much clearer effects than the overall shift of tumour incidence between animals irradiated at various ages.

322. Baserga et al. [B36] studied tumour induction in CxA F_1 mice after exposure to radioactively labelled thymidine. Variables analysed were: the age of the animals at exposure (including 15-17 days p.c. fetuses, newborn animals and adults at 2, 6 and 12-14 months of age): the magnitude of the thymidine dose and the manner of dose fractionation (between about 7.5 and 370 kBq per gram body weight delivered as single doses or in 6 fractions over 8 days); and the effect of ^{14}C-thymidine, as compared to ^{3}H-thymidine. Only malignant tumours were considered and there were no appreciable differences between mortality rate and tumour incidence between the two sexes. The data showed that both types of thymidine produced shortening of life span and an excess of tumours; that tumour incidence was dose-related and greater in animals injected as young adults than later in life; and that tumour incidence was also significantly above the spontaneous level in animals treated in the fetal stage. The small number of tumours seen, and the fact that the experiments reported did not extend until spontaneous death of the animals, preclude any meaningful comparison of tumour incidence between different ages at irradiation in this series.

323. The leukaemogenic effects of ^{32}P given intraperitoneally as $NaPO_4$ were studied in 3-5-months-old BALB female mice and in animals of both sexes between 11 and 15 days p.c. [H17]. After contamination, animals were observed until spontaneous death or were killed when leukaemia was diagnosed; in any case the experiments were terminated when the animals were 2 years old. Autopsy examinations were performed and leukaemia (including leukaemia proper, lymphosarcoma and reticulum cell sarcoma) was diagnosed microscopically. Lack of separation between various types of leukaemias diminishes the usefulness of this study. In adult females, a significantly elevated incidence of leukaemia was seen, but the dose depen-

dence over the range of activities of ^{32}P administered (about 1.5 to 4.5 MBq per animal) was not significant. In females exposed as fetuses the incidence of leukaemia was also significantly increased, and the females appeared definitely more susceptible than males. Furthermore, there was a negative correlation with the dose in females (over the range of about 90 kBq to 3.3 MBq per animal), while in males no such correlation was documented. These differences were attributed to the fact that ^{32}P at the doses given affects ovarian development and disturbs an interaction with oestrogens in leukaemogenesis. Comparison of the incidence of leukaemia showed that female animals treated as adults were approximately as susceptible as when injected as fetuses.

324. Between the years 1971 and 1982, Rönnbäck published a series of papers [R4, R10-R14] (also collected and jointly commented in a thesis [R5]) in which he described the effects of ^{90}Sr irradiation on the fetal ovaries of CBA mice. In none of these papers was there information on the actual doses absorbed in the fetal ovaries. However, it has recently been estimated [R26] that the effects of 11.1 kBq ^{90}Sr given to the pregnant mother would correspond approximately to those seen after doses to fetal ovaries of 0.010-0.013 Gy, given continuously over 4 days, from the 18th day of gestation to the 2nd day of extra-uterine life.

325. In short, it is shown in this series of experiments [R5] that the time for administration of the radionuclide to the pregnant mothers is a very important factor in determining the final degree of injury to the ovaries of the fetuses. When the number of germ cells remaining in the ovaries was scored at 56 days of extra-uterine age, it was found that the later during pregnancy that ^{90}Sr had been administered, the worst was the degree of injury to be seen, the maximum of effect being found for contamination at 19 days p.c. The earliest maturation stages, i.e., the "naked" oocytes, were in all cases the most sensitive cells. An activity-effect relationship was established in the range of 11-370 kBq ^{90}Sr given to the pregnant mother, and it was found that the fraction of germ cells remaining in the ovaries decreased linearly as a function of log-activity. The slope of the curve, however, remained the same, whether the damage was scored at 28, 56 or 84 days of age. Considering that there is a pronounced natural decrease of the number of oocytes as a function of age in untreated control mice, this observation indicates that the rate (as a function of dose) at which the germ cells decrease in the irradiated ovaries is independent of their initial number. A statistically significant reduction of naked oocytes was seen in these experiments after about 11 kBq ^{90}Sr.

326. In parallel with the short-term morphologically recognizable damage, long-term functional and carcinogenic effects were also assessed in these experiments by testing the reproductive capacity and the incidence of ovarian tumours in the mice contaminated in utero. Reproductive performance over a period of 7 months was tested by standard methods over the range of 46-740 kBq ^{90}Sr given to 19-day pregnant mothers. Only starting from 320 kBq were there signs of impaired fertility. The signs were quite obvious at 740 kBq, the rate of pregnancy being significantly reduced. The total number of litters and of offspring per female, and the reproductive period, started to decline at 185 kBq and were significantly affected at 370 kBq. It should be noted, for the sake of comparison, that at 185 kBq only 25% of the oocytes and follicles remained in the ovaries at the beginning of the mating period and that, 7 months later, at the end of it, the ovaries were essentially depleted of germ cells.

327. Tumour incidence was observed in the ovaries of 10-month-old animals that had been contaminated as fetuses with ^{90}Sr activities in the range of 46-740 kBq. Eighty-three out of 104 animals showed interstitial fibrosis, cysts and proliferation of the germinal epithelium. Different types of ovarian tumours (1 granulosa cell, 2 cystic adenomas, 18 tubular adenomas) were also found in these animals. There was a direct relationship between the frequency of animals showing proliferation and "down growth" of the germinal epithelium into the ovarian parenchyma and the frequency of animals having tubular adenomas, but tumours were not found until about 14% of the animals had shown downgrowths. Stimulation of the follicle-depleted gonads by gonadotropic hormones might be an important factor in the proliferative phenomena leading ultimately to the production of tumours.

328. Another paper by Walinder and Rönnbäck [W30] added more information on the induction of thyroid and pulmonary tumours in CBA mice, given pre-natally ^{131}I and whole-body x irradiation at 18-19 days of gestation. This paper gave emphasis to the role of cellular proliferation as a tumour-enhancing factor, particularly by comparison with proliferation in the adult mouse. It confirmed that tumour frequency was higher and latency shorter in the thyroid of the pre-natally irradiated animals than in the adults. Lung tumours were not more frequent in pre-natally irradiated mice than in non-irradiated ones, although their latent period was significantly shorter. Cell killing was found to be more pronounced in the thyroids of mice irradiated as adults than in animals exposed to the same doses in their fetal life. Therefore, the loss of cells' reproductive capacity leading to a lower tumour incidence was held to be less important for pre- and neo-natal thyroid carcinogenesis, than for carcinogenesis in mature glands. It was pointed out, however, that many other factors, in addition to cell proliferation, may determine the final incidence of tumours: for example, cells in the organs of the fetal and the adult animal could be at various stages of differentiation. This condition, among others, would of course make comparisons across ages extremely difficult.

2. The rat

329. Sikov and Lofstrom [S13, S46] treated Sprague-Dawley rats with x rays (20 or 100 R on day 10 and 50 or 185 R on day 15 of gestation). Later in the animals' life they observed decreases in growth and longevity that were exposure-related. The incidence of mammary

tumours in female animals given the highest exposure at 15 days was decreased. However, there was a shortening of the latent period in tumours developing in irradiated rats, as opposed to those appearing in the controls. An exposure of 50 R produced a tumour rate almost identical with that in the controls, with a greater tendency towards the presence of multiple tumours in tumour-bearing animals. Tumours of other sites were not appreciably increased. After 185 R, the gonads were atrophic, and this suggested an association between the decreased incidence of mammary tumours and the ovarian changes at this level of exposure.

330. Wegner and Graul [W17] irradiated (180-kV x rays, 1 Gy) Wistar rats in utero on the 9th day p.c. and followed them after birth for the appearance of malformations and tumours. About 40% of the animals showed brain or eye malformations and 10% developed tumours (as compared with a spontaneous rate of 3.4%) mostly of the malignant type, although benign mammary tumours were also observed. Animals bred through the second generation had no malformations, no significant decrease of life expectancy and no increased tumour incidence. In other experiments [W18], animals of the same strain were exposed (270 R) on the 18th day p.c. There was a high incidence of microcephaly and microophthalmia and genital hypoplasia in both sexes. In males, the incidence of tumours (total and malignant) was increased significantly above the control level, but it appeared to be slightly lower in these animals than in other animals exposed post-natally. In females, the incidence of mammary tumours was markedly decreased by pre-natal exposure, while it increased in rats exposed after birth. Other tumours of the genital tract (uterus, ovary) were reduced by pre-natal as compared with post-natal exposure. When all genital tumours were excluded, the incidence of other malignancies was slightly greater in females exposed post-natally than pre-natally.

331. The experiments by Strel'tsova and Pavlenko-Mikhailov [S48] were carried out on outbred white rats irradiated during the pre-implantation period (7 days p.c., 240 animals), during late organogenesis (14 days p.c., 105 animals) and during the fetal period (19 days p.c., 120 animals). The methods for counting stages in development are not specified in the paper. A single dose of 1 Gy of ^{60}Co gamma rays was administered in all cases. The animals, together with some controls, were followed in time to study tumour appearance. The results reported refer to 219 test and 53 control animals dying between 200 and 600 days of age. About 17% of the control animals developed tumours during this time, as compared with about 65% of the animals irradiated during early organogenesis, late organogenesis (63%) and the fetal period (43%). Animals irradiated at 14 days p.c. developed tumours up to 400 days of life, while in the other two test groups tumours only appeared between 120 and 180 days of life. Male animals irradiated at 7 days p.c. showed tumours of the mammary gland and bones, while the females irradiated at the same time showed ovarian and bone tumours, which were not observed in control animals.

332. Kalter et al. published a series of papers on the interaction of ethylnitroso-urea (ENU) and x rays administered in utero in respect of the oncogenic response of rats. In a first series of experiments [W7] pregnant Sprague Dawley rats received 2 Gy of x irradiation on days 15 or 16 p.c., and 10 mg/kg ENU one to four days later. Controls only irradiated or only injected with ENU were also set up. By the 15th month post-exposure, about 17% of the offspring exposed to both agents pre-natally developed neurogenic tumours, as opposed to 62% of those exposed to ENU alone, or to no tumours at all in those exposed only to radiation. The first signs of tumours appeared later in animals exposed to the combined treatment. In order to probe deeper into this antagonistic action, in a second experimental series [K1, K2], rats were irradiated on day 16 with a range of doses from 0.05 to 2.5 Gy and received ENU (10 mg/kg intraperitoneally) on day 20 of gestation. The frequency of animals surviving beyond 4 weeks of age that developed tumours in the course of their entire life span was inversely related to the dose of x rays. Thus, ENU alone caused about 69% of animals to develop tumours; 0.25 Gy + ENU, 46%; and 2 Gy + ENU, 15%. The mean time for the appearance of neurogenic tumours and the mean number of tumours for each affected animal were unrelated to tumour frequency. Analysis of the rate of tumour appearance indicated that the initiation, rather than the promotion, stages of tumour induction could be responsible for the reduced frequency. Killing of target cells, probably glioblasts, was not in itself entirely sufficient to explain the results.

333. The subject of the interaction between radiation and ENU was also taken up in another paper by Schmahl and Kriegel [S82]. In their experiments, single and combined treatments were administered to Wistar rats with the chemical alone or after x-ray doses of 0.5-1.5 Gy. Animals were in the 13th day p.c., at which time both agents may induce brain malformations and ENU, alone or in combination, may produce brain tumours. The possible correlations between the teratological and the carcinogenic endpoints were investigated by a histopathological analysis of the type and degree of malformations, and the distribution patterns of tumours in the forebrain. The existence of a correlation was shown not only by the simultaneous occurrence of both effects in the same brains, but also by a higher tumour multiplicity (mostly gliomas) in the malformed brains and their preferential location in the subependymal layer, which was also the most heavily malformed structure. Another effect of irradiation, the occurrence of heterotopic neuronal nodules called "rosettes", which is only apparent at doses above 1 Gy, correlated negatively, however, with the occurrence of gliomas. In view of the authors, this explains the substantially decreased incidence of gliomas following combined treatments involving doses in excess of 1.0 Gy, and also the consistent multiplicity of tumours, in spite of their decreased frequency.

334. Cahill and his group studied neoplastic and life-span effect after chronic exposure to tritium. In a first paper [C1], Sprague-Dawley rats were exposed to equilibrium levels of tritiated water during pregnancy

and up to parturition. Four different levels of the radionuclide resulted in cumulative whole-body doses of about 0.066, 0.66, 3.3 and 6.6 Gy. The animals at the two higher dose levels showed, throughout their life span, an increased incidence of mammary fibroadenomas. Data were not such as to allow the definition of a precise dose-response curve; they were, however, not incompatible with linearity. Increasing doses of radiation shortened the time for the onset of mammary fibroadenoma. A second paper [C2] reported that the offspring of the animals exposed in utero to doses up to 0.66 Gy showed no effect with respect to the overall incidence of neoplasia, to incidence rate, or to age at onset of mammary fibroadenomas. Females exposed to 3.3 or 6.6 Gy were sterile and had lower rates of mammary fibroadenomas than did controls. At 6.6 Gy females showed a lower incidence of overall tumours. Regardless of dose, females had a significantly higher incidence of neoplasia and a longer life span than males.

335. In another set of experiments, Berry et al. [B37] attempted to induce bone tumours in the offspring of Sprague-Dawley rats by treating pregnant mothers (16, 18 or 20 days p.c.) with ^{32}P (about 37 or 110 kBq per gram body weight). Fetal tissues were judged to be resistant to irradiation since no increased frequency of tumour was found in the treated animals. However, naturally-occurring tumours showed a clear tendency to appear earlier in post-natal life in the treated animals. On this basis, it was concluded that transplacental exposure of rats to ^{32}P provided no evidence of enhanced oncogenic effects in the fetus.

336. Other experiments were reported by Schmahl and Kollmer [S31, S64] on the carcinogenic effect of ^{90}Sr injected into pregnant rats at 18 days p.c. (3.7 or 5.5 MBq per rat). The radionuclide accumulated in the ossification centres of the skull of fetal animals to deliver a dose which was calculated to be between 0.6 and 1.2 Gy (over the entire life span) within the bone surfaces. Approximately 50% of this dose was delivered within a week of the injection of the nuclide. The offspring of the treated animals were examined at autopsy and pathologically. They had an incidence of pituitary tumours which was, as compared with non-treated control animals, about 10 times higher in the males and three times higher in the females. In about 6% of treated animals, metastasizing meningeal sarcomas were seen, and in 2%, pituitary adenomas and meningeal sarcomas occurred simultaneously.

337. Sikov et al. [S47, S54, M40] injected intravenously into adult, weanling, new-born and 19-days-p.c. rats ^{239}Pu cytrate in different amounts, adjusted in such a way as to deliver radiation doses of approximately 0.07, 0.23 and 0.7 Gy to these animals' femurs during the first 10 days after injection. Doses to the various age groups were markedly different. Most of the dose to the younger animals was absorbed within the first few months. Weanlings and adults received a more prolonged and substantially higher cumulative exposure. The animals were observed until their natural death, but the experiment was terminated after 30 months. The survival times of rats exposed post-natally became progressively shorter as the dose increased, but no life shortening was seen in animals exposed pre-natally. Bone tumour incidence increased in all exposed groups, irrespective of age. Younger rats appeared to be less sensitive on an administered-dose basis, but were perhaps more sensitive on a cumulative-dose basis. A biphasic cumulative dose-incidence relationship with a maximum at about 0.25 Gy and a drop at 0.8 Gy was seen in animals exposed pre-natally, but it is not clear how much of this phenomenon may be true or spurious (for example, owing to confounding maternal effects). Pre-natally exposed rats cross-fostered to non-injected females, in order to ensure that they would only be exposed while in utero, had the highest incidence of bone tumours, even higher than that of rats remaining with the injected mothers. This result was probably related to the high dose given to the dams, which affected their capacity to care for the offspring. The site of radiation-induced tumours also varied as a function of age: in the younger animals, most tumours arose in the head or in the vertebrae; in the adults and weanlings, they were mostly in the extremities, with only a few in the vertebrae or other places. It was concluded that the younger animals were probably more susceptible per unit dose than the adults. It is clear, however, that this susceptibility is also a function of the rate of dose delivery and therefore is not easily defined.

338. Two abstracts reported on the late effects of ^{131}I in Fisher rats. The first [C20] regarded animals exposed in utero to concentrations of $Na^{131}I$ ranging from 4 kBq to 3.7 MBq during days 16-18 of pregnancy. Two months post-partum, the animals showed an alteration of immunological reactions (cell-mediated immune response and antibody-dependent cell-mediated cytotoxic response) to the carcinogen 1,2-dimethyl-hydrazine, suggesting that the original perinatal exposure produced a persistent altered reaction to the chemical. The second abstract [L11] reported on a higher immunological response of the male animals exposed in utero, which was 1.7 times that of the female offspring.

339. Sikov et al. [S80] exposed groups of 50 to 80 pre-natal, new-born, weanling and adult rats to various levels of ^{131}I and scored their thyroids microscopically at death or at 30 months of age for the presence of tumours. These were commonly C-cell type in control animals and follicular in the exposed ones. Doses were estimated by radioanalysis in the thyroids of the various groups. When given post-natally the ^{131}I increased tumour incidence significantly in the range of 1 Gy, but led to a further decrease down to control levels after doses of 20 Gy or greater. When given pre-natally (from 17 days on), the ^{131}I produced a higher incidence of tumours at all dose levels of 0.4, 4.25 and 34 Gy. The form of the dose-response relationships was characterized by a peak at intermediate doses in the two older groups, but was progressively increasing with dose in the two groups exposed perinatally.

3. The dog

340. Preliminary data are also gradually becoming available for the dog irradiated in utero. In one report

[P7]. 4 groups of 120 beagles were irradiated with gamma rays from ^{60}Co in the late fetal period (55 days p.c.) or soon after birth (2 days of age) with 20 or 100 R delivered over a constant time of 10 minutes. The morbidity and mortality of these animals was followed over 2 years and compared with that of 450 control dogs and of other dogs exposed to the same dose levels at other ages. Three of the 240 dogs at the 100 R level and 1 of the 240 at the 20 R level died with tumours within the observation time, while in controls and animals exposed at other ages, no tumours were observed . No tumour would be expected to appear by chance in irradiated dogs.

341. An abstract published in 1978 [B4] updated the experience to 5 years post-exposure and concluded that of 20 dogs developing tumours, 19 had been irradiated. The experiments were not sufficiently advanced to draw significant conclusions as to the role of age as a factor in radiation injury. Another report [B28] gave evidence that the incidence of lymphoma in the irradiated dogs, especially those exposed at 55 days p.c., was considerably higher than that in the controls or than that expected in a random canine population. Final results of these series, as well as of another series on beagle dogs receiving continuous whole-body irradiation [S29, S30], will not be available for a few years.

342. Experiments by Momeni [M41] were aimed at studying the incidence of bone sarcoma and myeloproliferative disorders as a function of dose rate and time in beagle dogs fed diets containing $^{90}SrCl$ (in equilibrium with ^{90}Y) from mid-gestation to 1.5 years of age. Median incidence time and standard deviation of the distribution were calculated for the tumours at each level of administered activity. For primary osteosarcoma, this time increased from 2.8 years of age for animals receiving 1.3 MBq ^{90}Sr/day to 12.6 years for those receiving about 1.50 kBq ^{90}Sr/day. Although very valuable for extrapolation of the carcinogenic effect to low doses and dose rates, these data do not lend themselves to a comparison of susceptibility to tumour induction in the pre- as opposed to post-natal ages.

B. DATA FROM MAN

343. One of the most important sources of information about the induction of tumours in children irradiated pre-natally is the so-called Oxford Survey of Childhood Cancer. Data from this series were first reported in 1956 [S44] by Stewart and her colleagues. These authors confirmed in 1958 [S23] that the mothers of children who died from leukaemia or other malignant diseases remembered having been subjected to abdominal or pelvic x rays during the related pregnancy with a frequency that was higher than that of the mothers of control children who had not developed cancer. This finding stimulated much interest. For a description and history of the Oxford Survey see Steward [S22]. Soon other reports followed and the experience accumulated up to 1961 [F6, K21, K28, M39, P9, S23, S44, S45] was summarized by UNSCEAR [U3] as in Table 12.

344. In its 1964 report [U3] UNSCEAR reviewed other data that had appeared up to that time [L5, C12, M22, M25, W16]. It pointed out that, as established by MacMahon [M22], the joint maximum likelihood estimate of the relative risk factors in all the surveys reported was 1.4, with 95% confidence limits of 1.2 to 1.6. On that basis, UNSCEAR concluded that, although accurate dose estimates were not available, irradiation of fetal tissues could give rise to a greater risk per unit dose than post-natal irradiation, possibly by a factor as high as 5. It pointed out at the same time, however, that it was difficult to separate such leukaemogenic effect of radiation from other possible etiologic factors connected with the reason that had prompted the radiological examination of the conceptus in utero.

345. Further data on the carcinogenic risk of irradiation in utero were reviewed again in the 1972 UNSCEAR report [U4]. The papers by Graham et al. [G11], Jablon and Kato [J4] and Stewart and Kneale [S24, S26] were specifically examined. The report drew attention to the discrepancy between the negative data from Hiroshima and Nagasaki and those from irradiations performed for medical reasons. It also discussed the difficulties of deriving precise estimates of risk from cohort (rather than from case control) studies, because the cancer risk in children less than 10 years of age is rare (of the order of 10^{-4}) and cohort studies of sufficient size are therefore difficult to carry out. The report concluded that children born of mothers irradiated during pregnancy seemed to have an increased risk of cancer in their early age. The estimate put forward at the time, most likely in excess, was of the order of $2\ 10^{-3}\ Gy^{-1}\ a^{-1}$ over a 10-year period, in the range of 0.002 to 0.2 Gy. However, the possibility could not be ruled out that the associations found (or at least part of them) could be related to factors other than irradiation itself.

346. In 1977, UNSCEAR again examined the problem of the carcinogenic risk of irradiation in utero, on the basis of other data by Mole [M23], Newcombe and MacGregor [N3], Holford [H16], Stewart and Kneale [S24], Jablon and Kato [J4], and Kato [K20]. The analysis led to the conclusion that the risk, per unit absorbed dose, of fatal malignancies induced by fetal irradiation could be in the region of $2\text{-}2.5\ 10^{-2}\ Sv^{-1}$. Half of these malignancies could be attributed to leukaemia and one quarter to tumours of the nervous system.

347. Subsequently, the Committee on the Biological Effects of Ionizing Radiation (BEIR) [C13] examined the literature available up to 1980 on the subject of radiation carcinogenesis in utero. In essence, it concluded that an association existed between intrauterine irradiation and childhood cancer, but it recognized that such an association could be attributed, to some extent, to factors involved in the selection process for submitting the pregnant mothers to radiodiagnostic examinations. Strong support for a causal relationship came from twin data from the Oxford series, while weak support for a selection effect came from the ascertained relationship between pre-natal irradiation and neo-natal health and from

the relationship between neo-natal health and later, clinically detectable cancer. The BEIR Committee, on the basis of adjusted data from the Oxford Survey estimated the relative risk of cancer after exposure in utero relative to irradiation after birth to be 5.0 for the first-trimester exposure and about 1.5 for the remaining intra-uterine life. The period at risk appeared to be within the first 12 years of post-natal life for haemopoietic tumours and within 10 years for other solid tumours. The risk coefficients were estimated to be at 2500 excess fatal leukaemias and 2800 excess fatal cancers of other types per million children, year and gray.

348. MacMahon [M52] later reviewed the conclusions of the BEIR Committee report on this subject, adding some useful considerations. According to this author, there is no doubt about the existence of a relationship between exposure in utero and subsequent likelihood of developing a malignancy within the first 10 years of life. The relative risk associated with prenatal exposure is of the order of 40 to 50% over non-exposed children. A number of variables (medical, sociological, methodological) have clearly been identified which tend to confound the association, but even correcting the data on their account would not be sufficient to justify the association quantitatively. The most difficult problem is that of establishing whether the association is causal or by chance. On the one hand, it is difficult to prove that this may not have been due to characteristics of the mothers, the children, or the pregnancy, that may also have been associated with a tendency of the children to develop a malignancy. On the other hand, no such characteristics have been found. There is, in favour of a direct causality, the observation of Mole [M23] on single and twin pregnancies, but this is based on a re-interpretation of the same series. Against a causal relationship there are, however, cogent data from Hiroshima and Nagasaki and from experimental animals. According to MacMahon [M52] it is biologically implausible that fetal doses in the region of a small fraction of a Gy should increase the relative cancer risk by 40 or 50% within 10 years of age while doses in excess of 1 Gy should fail to do so over longer periods of time, as pointed out by Jablon and Kato [J4]. There is, further, [M52], the constancy of the relative risk over all categories of malignancy, which is not in agreement with evidence on radiation carcinogenesis [U2] indicating a remarkable degree of variability between tissues and ages in their response to radiation. Finally, on the question of the alleged dose-response relationship, MacMahon [M52] argues that data analysis as a function of dose is based on the acceptance of the causal nature of the association, which he is inclined to doubt in the case at hand. Given the difficulty of finding new material for independent analyses, MacMahon is also inclined to regard this issue, important as it may be, as an unresolved curiosity which will gradually fade into medical history, giving rise, in the process, to more confusion than understanding.

349. The references given thus far, on which previous conclusions have in large part been founded, are the most relevant to the point at issue but they by no means exhaust the long list of papers published on this subject, even before the last UNSCEAR review. Other references should also be kept in mind to complete the publications available up to about 1977 [A9, B11, B22, B32, D14, D15, G2, G10, G12, J8, L6, M26, O5, S22, S25, S39, S41, S42, W15]. What follows updates information published since then.

1. Atomic bomb survivors

350. The most recent report on the mortality experience of children exposed in utero to the atomic bombs refers to the period 1945-1976 [K4]. The study sample included 1293 children (1074 exposed at Hiroshima and 219 at Nagasaki) whose status and causes of death were obtained from Japanese family records and death certificates. Doses in utero were estimated by two different groups: one at the Oak Ridge National Laboratory in the United States, the other at the National Institute of Radiological Sciences in Chiba, Japan. The methods used by the two groups were slightly different, but the tissue doses obtained were almost identical and correlated well with the T65 dose, although they were lower by about 40%. (These doses will, however, be subject to revision at some later stage.) Total deaths up to 1976 numbered 203, and the mortality ratio increased linearly with T65 and tissue dose in both cities. The dose-related mortality ratio varied, however, with age at death: it increased with dose for those dying under 1 year of age, particularly for those dying within 1 month of birth; it showed no increase at 1-9 years of age; and an increase was again suggested after 10 years of age. Analysis of the data as a function of trimester of exposure showed a significant increase of mortality ratio with dose only for children exposed in the third trimester.

351. Mechanical injury received by the mother was insufficient to provide an explanation for the excess in early infant death. Birth order and birth weight could not have seriously affected the relationship between mortality and exposure. Precise information about the causes of death was not available for 55 of the 203 deaths, and these 55 occurred almost exclusively within a year of birth. This circumstance did not allow a fine breakdown of causes of death. The following, however, were analysed: perinatal death, which was found to increase with dose; and cancer death (2 leukaemias and 3 solid tumours of the digestive organs), which was not found to be related to dose, even when additional samples defined only in 1960 were analysed. There was no statistically significant evidence of an excess in cancer death among individuals irradiated in utero. However, the sample size and the number of deaths were too small to reach a firm conclusion.

352. A further report by Ishimaru et al. [I3] is of relevance. These authors studied the incidence of leukaemia in a sample of 3636 children (including controls and exposed in utero) in the two cities. Exposed were those whose fetal doses were between 0.01 and 0.49, and in excess of 0.5 Gy. The crude annual incidence rates for exposed and controls in

both cities during 1945-1979 are shown in Table 13. During the 34-year follow-up period, 3 leukaemia cases were identified, 2 in the control groups, and 1 in the group exposed between 0.01 and 0.49 Gy. It was calculated that, in order to show any statistically significant effect of radiation, 7 cases of leukaemia would have to occur among the 705 irradiated subjects included in the sample. Thus, there appears to be no excess risk for the exposed children. This is in agreement with previous findings [J4, K4] in this series.

353. In 1974, Mole [M23] used a radiobiological argument to show that the discrepancy between the observations from Japanese children exposed to atomic radiation in utero and medically irradiated British children could be the result of an artefact, since the killing of potentially transformed cells, particularly at the highest doses, might be responsible for the low rate of tumour induction observed in the Japanese series. Using reports from the early 1970s [J4, K20], and correcting the tumour induction data according to an exponential cell killing function, Mole showed (under a number of qualifying assumptions) that the true induction rate per unit dose might have been three to four times higher than that actually found, thus bringing the results of the Japanese series much closer to those of the Oxford series. In the meantime, new information has been produced [K4] which extends the observation period up to 1976 in Japan, always with negative conclusions. Mole's reasoning was also based on old dosimetric data that are currently under revision. His conclusions, therefore, will have to be verified against the new dosimetry (particularly the doses to the fetus) to see whether they retain their validity.

2. Medical exposures

354. The present study reviews only the contributions that have appeared since the last UNSCEAR report on the subject [U2]. Kneale and Stewart returned to the analysis of the Oxford Survey with two more papers published in 1976. The object of the first paper [K9] was to discover whether the excess of fetal irradiation histories associated with development of early neoplasms could be an artefact due to the association between obstetric radiography and other birth factors of etiologic importance. To this end the authors applied the Mantel-Haenszel procedure, which permits identification of separate effects of associated factors in retrospective surveys, to analyse the effects on childhood cancer of social class, maternal age, birth order, and fetal irradiation. A further objective was to test whether these conditions might also have similar effects on other childhood cancers than reticulo-endothelial-system (RES) neoplasms. The new analysis showed that all these factors did exert independent effects on RES neoplasms and all, except sibship position, independently influenced cancers other than leukaemia. However, the degree of these joint associations could not account for the fact that radiation exposure in utero, under the conditions tested, is associated with a higher risk of developing juvenile cancer.

355. The second paper [K19] addressed several different points and led to a number of conclusions. There was no statistically significant proof of a dose-effect relationship, but only suggestions, that the risk of developing juvenile cancer did increase in proportion to the number of radiographic films used. In view of the retrospective character of the survey, of the variability in the number of films used and their exposure in different places and over a long period, this evidence may not be very reliable. There was also a suggestion of a higher susceptibility to cancer induction for children irradiated in the first trimester. There were relatively fewer cancer cases among children with abnormal radiological findings than among other x-rayed children, owing, presumably, to a positive association between several ante-natal conditions and early non-cancer death. There were also relatively fewer cancer cases for recent than for remote exposures, probably due to a progressive lowering of film doses between 1940 and the time of reporting.

356. In 1981, Totter and MacPherson [T2] published a critical review of the data in the Oxford Survey, in order to re-examine the evidence in favour of a causal relationship between fetal irradiation and cancer development. This review was based on a number of arguments. First, the authors showed that the greater frequency of x-ray examinations reported among the cancer cases than among the non-cancer cases decreased with time between 1946 and 1963. This observation had been made before by others [B22, S24, S26], but, according to Totter and MacPherson [T2], had not been given the appropriate weight. In particular, the alleged risk had not been placed in a context relative to the natural risk of cancer induction. Secondly, the authors pointed out the striking discrepancy between the Oxford experience and that of Hiroshima and Nagasaki, also repeatedly noted in the past [J4, K4] and confirmed by the most recent findings [I3, K4].

357. Thirdly, Totter and MacPherson [T2] criticized the method used in Stewart's work of separating the "radiogenic" and "idiopathic" cases of cancer from the overall cancer cases. In their view, this method assumes that the idiopathic cases have the same likelihood of being irradiated as the general population and, therefore, as the control. Such an assumption would be acceptable if it could be shown that: (a) the irradiation of the mothers took place at random; and (b) the characteristics of the idiopathic cases were sufficiently close to those of the control population. In order to test for (a), irradiated and non-irradiated controls were compared for a number of traits (described in [B22]), adjusted for the purpose of identifying the mothers most likely to have problems during pregnancy or selecting the most affluent families. The analysis showed consistent differences among the irradiated and non-irradiated controls treated as two populations. From these, Totter and MacPherson concluded that the choice of irradiating the pregnant mothers was not made at random. To test for (b), the cases developing tumours were compared with tumour-free controls and then shown to be significantly different from the controls in respect of a variety of population characteristics (social class, urban or rural residence, sibship, age and

medical history of the mother, etc.). The differences were thought to be significant and taken as evidence against the assumption that the idiopathic cases would be irradiated with the same frequency as control cases.

358. Fourthly, Totter and MacPherson [T2] criticized the dose-response curves of Stewart [B22, S24] that show an excess risk of childhood malignancy, increasing as a function of the number of films used in the pre-natal x-ray examinations. They pointed out that the Oxford series only demonstrated a difference in frequency of pre-natal x rays between the groups developing and not developing cancer. Under such circumstances, the greater risk caused by the irradiation procedure may only be inferred on the unproven assumption that the normal x-ray frequency in the cancer group is the same as is the non-cancer one. Furthermore, the notion of excess risk as defined by Stewart (the relative risk minus 1), presupposes zero risk for zero dose and therefore assumes that any value greater than zero for each number of x-ray films may be attributed to the x-ray exposure. Finally, the excess risk in Stewart's work is not related to dose, but to the number of films used in the x-ray examination, which is not a good measure of dose because this number was never known with any accuracy. Furthermore, the dose per film varied consistently with time and, therefore, by pooling exposures received at different dates, different doses per film would be indiscriminately mixed.

359. In an expanded treatment of the Oxford data published in another paper [T6], Totter showed that the dose-response relationship claimed by Stewart and Kneale [S24] could be duplicated or improved upon by substituting random numbers in place of the actual numbers found in the survey. According to Totter [T6], the claimed dose-response relationship would be the result of a statistical fault in the use of "excess risk", due to the fact that the highest exposures are received by the smallest number of persons. Therefore, data points with the greatest relative error are multiplied by the reciprocal of the lowest frequency which is itself expected to have a large error. The numbers resulting would thus carry the greatest, and not the least, weight in an ordinary regression analysis.

360. Commenting on the correlation—as opposed to causation—between in utero exposure and subsequent increased risk of malignancy, Burch [B13] pointed out the impossibility of excluding that the medical conditions prompting the selection of cases to undergo obstetric radiology might themselves be associated with an enhanced occurrence of childhood malignancy. He stressed the lack of supporting evidence from the Hiroshima and Nagasaki studies, and pointed out that an independent analysis of the age of onset of childhood malignancies, as found in the Oxford series [B34], cast doubt on the validity of the causal association.

361. Bross's hypothesis, that leukaemia risk from obstetric radiology could be increased by the presence of genetic factors that also enhanced the risk of certain indicator diseases, was tested on the Oxford childhood cancer cases by Kneale and Stewart [K10] who analysed the interaction of pre-natal irradiation and post-natal diseases in children developing tumours. The authors studied about 6000 cases born between 1953 and 1962 who died before 12 years of age, together with matched controls. Reticular system neoplasms (3199 cases) and other solid tumours (2803 cases) were studied, together with matched controls (6002). Those that had developed a serious infection prior to death were evaluated separately. The data showed that there was a significant association (at the 5% level) between the two variables (cancer and infection) in those which were x-rayed, but not in the non-irradiated controls. Respiratory infections (pneumonia, bronchitis, tonsillitis) were the diseases mainly responsible for the effect. The data are compatible with both the hypothesis of an early damage to the immune system caused by radiation, or that of an earlier origin and termination of the non-irradiated than irradiated cancer cases. Contrary to Bross' interpretation, it is suggested that the relatively low frequency of infections among the non-x-rayed cases might be an artefact caused by an earlier development of immune system faults in the x-irradiated cases.

362. In a short letter [B35], Burch commented on Mole's findings [M23] that there is a similar excess of leukaemia and solid cancers in singleton and twin births, in spite of their different rates of radiological examination. He pointed out that calculations of death rates for leukaemia and solid tumours during the first 10 years of life, combining irradiated and non-irradiated singletons, on the one hand, and irradiated and non-irradiated twins, on the other, result in a slightly, but not significantly, lower rate in the latter. Considering that 50% of twins were irradiated in utero (as opposed to only 10% of singletons), which would lead one to expect a 20% higher rate in twins, the finding cannot easily be reconciled with the view that tumour incidence is causally related to radiation exposure. Burch further pointed out that Mole's estimates [M23] are affected by such wide error limits that they cannot legitimately be used to support the idea of the causal relationship. He concluded, therefore, that, although it may be prudent to act as though ante-natal exposure may increase the carcinogenic risk, the validity of the phenomenon has not been scientifically proved.

363. Einhorn [E6] observed that cell proliferation and differentiation are most active during embryonic life, but cancer is rarely seen to occur after irradiation at these stages. On the other hand, human embryos become susceptible to tumour induction after the period of major organogenesis, particularly in those tissues that have a late development and are still immature at birth. The author suggested that these phenomena might be explained on the assumption that the regulators influencing development, which are most active during the embryonic life, might be the same ones that control cancer induction during that developmental period.

364. In its 1964 report [U3] UNSCEAR already reviewed a study by MacMahon [M25] on 734,243 children born and discharged alive in 1947-1954 in 37

maternity hospitals in the north-east areas of the United States. The frequency of intra-uterine exposure of the entire child population was estimated by reviewing the records of a 1% systematic sample (7242 singleton children). Abdominal or pelvic irradiation was found to have taken place in 770 of these children's mothers, corresponding to 10.6% of the reviewed sample. It was ascertained, by review of death and birth certificates, that 584 children born in the study population had subsequently died of cancer. The pregnancy and delivery records of 569 cases were inspected. Out of 556 children dying of cancer that had been born of single pregnancies, 85 (15.3%) had been exposed in utero to diagnostic x rays. The higher frequency of pre-natal x rays in these cancer cases, compared with the rest of the sample (10.6%), was found to be statistically significant. Data were corrected for indirect associations with birth order and other complicating variables, and the final adjusted cancer mortality risk was found to be about 40% higher in the in-utero-irradiated than in the non-irradiated members of the test population. The excess of neoplasms found applied equally to leukaemia, tumours of the CNS and other neoplasms. The excess cancer mortality of the exposed children was most marked at the ages of five to seven years (with a relative risk of about 2.0) and apparently exhausted by the age of 8 years. There was a small non-significant trend toward a higher mortality in the most heavily irradiated children, but no significant variation of the risk was found with the intra-uterine age of children.

365. Monson and MacMahon [M53] reported recently on an extension of their previous study [M25]. The new paper includes data for five additional hospitals and extends the period of coverage of birth and death. It is based on cancer deaths occurring between 1947 and 1967 among 1,429,400 children born between 1947 and 1960 in 42 maternity hospitals. It excludes children born of multiple pregnancies or dead in the delivery hospitals. Out of this study sample, through a search of death certificates, 1342 children were identified who died from cancer before the age of 20. The birth records of these children, and of a 1% sample of the total population, were reviewed in order to determine which of the children had received irradiation in utero through irradiation of the mother's abdominal region. Since pre-natal x-ray exposure may be associated with many causes of childhood death, in addition to cancer death, the authors followed the x-ray experience of other children who died from causes other than cancer. This control sample included children born in 17 hospitals during the study period, who died after 3 months of age in 1950, 1955, 1960 or 1965. The risk ratio for leukaemia between the 1% sample and the study sample was 1.48, that is, very similar to the 1.5 found in the earlier analysis [M25]. On the other hand, the risk ratios for cancer of the CNS and other cancers were appreciably lower (1.16 and 1.14) by comparison with those found in the previous study (respectively, 1.6 and 1.4). In view of the similarity of the risk ratios, all solid tumours were combined in the subsequent analysis.

366. The excess of leukaemia cases in children with a history of x-ray exposure (over the selected cases based on the 1% sample) was only evident between 2 and 7 years from birth, whereas the excess of solid tumours occurred between 2 and 9 years. Thus, further analysis was limited only to children who died before their 10th birthday. The maximum likelihood risk ratio (MLR) for leukaemia was essentially the same in the initial study (1.48) [M25] and in the extension (1.58) [M53]. For solid tumours, the MLR was 1.45 in the initial study and 1.06 in the extension. The overall total MLR for leukaemia of 1.52 and for solid tumours of 1.27 are not significantly different when directly compared ($P = 0.4$). In order to evaluate other possible confounding factors, the frequency of x-ray exposure of children in the 1% sample was examined according to selected characteristics, together with the incidence rate of cancer for the same characteristics. This analysis showed that factors such as birth order, sex, birth weight, year of birth and abnormality of pregnancy (acting alone or in association) could confound the correlation between x rays and cancer, being associated with both in the same direction. On the other hand, race, pay status and previous fetal loss were potential negative confounding variables, in the sense that children with more exposure had developed less cancer. Maternal age and religion were not consistently associated with x-ray exposure or cancer risk and could not, therefore, have confounded the data.

367. Correction for each of the potential confounders brought about little change in the risk ratio. There was, at the same time, no indication that the overall association between cancer and x irradiation was present only in a limited sub-population of the whole sample. When the number of x-ray examinations observed among children with cancer was compared with those expected according to the year of birth, an excess of x rays was observed from 1947 through 1957 for leukaemia, but only for the period 1951-1954 for solid cancer. However, no reason for this clustering of the excess was identified. Children developing leukaemia did not appear to have received a higher rate of examination with x rays until the third trimester, but children developing other solid tumours had a higher rate of examination also during the first and second trimesters. Although no information was available on the actual doses received, there was no suggestion of a crude dose-response relationship, based on the type of examination.

368. From a discussion of possible sources of ascertainment bias, related to the facts that the record search was not blind and that there was some loss of information between the first and the second assessment, no evidence appeared that these conditions might have introduced any significant bias in the association between x-irradiation and cancer. It was concluded, therefore, that these new data were in overall agreement with other data pointing to a risk ratio of malignancy associated with pre-natal exposure to x rays of about 1.5. There is, therefore, no question in the mind of the authors as to the significance of their findings, but they believe that the relationship shown is not causal. This view is argued on the basis of the absence of confirmatory evidence from the experience of Hiroshima and Nagasaki, and from

experience in experimental animals. The authors point out that searches for variables that may have confounded the association have failed to identify any factor that might be of sufficient weight to explain the data. There may be, in principle, as suggested by Totter and MacPherson [T2], some kind of generalized greater access to medical care among fetuses that will eventually develop cancer, but such a hypothesis is difficult to test experimentally.

369. In a review article [D23], Doll outlined the main features of the Oxford Survey and discussed some of its difficulties. These included: (a) the possible bias introduced by incorrect reporting of previous x-ray history by the mother of a child who later developed cancer; and (b) the possibility that the association shown in the Survey might be a secondary one, due to the fact that some unidentified factor might be the cause of both the higher tendency to develop a tumour and of the higher probability of prenatal x-ray exposure. Doll was satisfied that the first issue was not likely to have influenced the results to any appreciable degree. As to the second one, although recognizing that it may be impossible to overcome it in any observational study, Doll believes that it is unlikely for three reasons: first, the failure to identify such a factor, in spite of intensive searching; second, the allegedly good relationship between number of x-ray exposures and the increase in excess risk (an argument which has, however, been challenged by Totter [T2, T6]); and, third, Mole's evidence on twins [M23] (although this, while in agreement with the later data of Harvey et al. [H23], is not accepted by MacMahon [M55] as a definitive proof).

370. Shiono et al. [S11] reported on a prospective study of 55,908 women participating in a collaborative perinatal project in the United States. They examined the x-ray exposure history of 145 mothers whose children developed neoplasms up to 7 years of age, and of 290 matched control mothers whose children developed no tumours. Of the 145 tumours under study, 40 were malignant and 105 benign. For x-ray exposure during pregnancy the relative risk values were 1.09 ($P = 0.526$) and 0.94 ($P = 0.635$) for malignant and benign tumours, respectively. Corresponding values for exposure before pregnancy were 2.61 ($P = 0.021$) and 1.07 ($P = 0.475$). For pre-conception exposure, there was no dose-response relationship of statistical significance in the groups of mothers whose children developed either benign or malignant tumours. For pre- and post-conception exposure combined, there was a suggestion that the relative risk could be higher for malignant neoplasms, but the significance of probability value was borderline. For benign tumours, the values of relative risk were not significantly different from 1.

371. Taken together, the data [S11] were thought to be consistent with an increased risk of malignant neoplasms among children whose mothers had been exposed to x rays before and during pregnancy, with a somewhat higher relative risk estimate for pre-conception exposure. There was no significant association between exposure and development of benign tumours. The data were interpreted according to the model of Knudson [K26], who postulated that certain tumours might be the results of two mutational events which could be in the form of either two somatic mutations or of one transmitted germinal mutation followed by one somatic mutation. According to this hypothesis, any agent that would increase the rate of mutation in germinal cells would be expected to enhance the risk of tumours. In addition, owing to the time factor involved in the accumulation of mutations, tumours (such as retinoblastoma) caused by a germinal plus somatic mutations would be expected to arise in early ages.

372. Salonen [S56] carried out a study on pre- and perinatal factors in childhood cancer, based on tumour registry data in Finland during the years 1959-1968. The immediately preceding baby born in the same maternal health centres from which the test cases had been drawn provided the control data. The number of test-control pairs of children included in the final analysis was 972. For all these pairs of children, information was sought concerning the parents, the pregnancy, the events at birth and the post-natal health, in order to compare the outcome of control and test children histories. Comparisons were carried out in terms of the total number of tumours and of leukaemias, brain and other tumours. There was no significant correlation between cancer and a number of potential etiological factors such as parental factors (ages of parents, mother's blood group, duration of marriage, social class of the family), pregnancy factors (numbers of deliveries or of pregnancies, pre- or post-mature delivery, use of drugs) or child factors (date of delivery, birth weight, vaccinations). As to leukaemia induction, the risk ratio in the group that had been irradiated in utero with pelvic radiology was 1.9, with an upper confidence limit of 6.7 at the 2% level. However, 5% of the mothers carrying children that developed leukaemia underwent pelvic radiography, as opposed to 3.7% of the mothers of non-leukaemic children. Therefore, owing to the rare occurrence of intra-uterine irradiation, the group was too small to render the increase of the risk ratio statistically significant.

373. A new study on childhood cancer in twins exposed pre-natally has recently been published [H23]. It is a case-control study, involving over 32,000 twins born in Connecticut, United States, from 1930 to 1969 and followed to 15 years of age. The twins were investigated for a possible relationship between prenatal x-ray exposure and childhood cancer and leukaemia. By concentrating on twins, rather than singleton births, it was hoped to reduce the chance of a medical selection bias, since twins were often exposed to x rays for diagnostic purposes, more so, in any case, than for any other medical condition. By matching the records of a twin and a tumour registry, 31 childhood cancers were selected for study (11 in boys and 20 in girls) and each of these cases was matched with 4 twin controls. Evidence for x-ray exposure and for other factors affecting pregnancy, delivery and maternal health was collected from a number of other sources. Abdominal exposure of the mother was particularly sought. It was estimated that the dose to the fetus from such procedures ranged

from 0.0016 to 0.04 Gy, with an average of 0.01 Gy per examination. All but one exposure occurred in the third trimester. The relative risk was taken as a measure of the association between radiation exposure and childhood cancer. Twelve out of 31 (39%) of the cases and 28 out of 109 controls (26%) were found to have been exposed pre-natally. The crude relative risk associated with pre-natal exposure was calculated to be 1.8 (0.8-4.2 were the 95% confidence limits). When adjusted for all the potentially confounding factors, the relative risk was 1.4-1.9. The adjusted risk for leukaemia was 1.6 (0.4-6.8) and for other childhood cancer 3.2 (0.9-10.7). In addition to pre-natal exposure, low birth weight was the only variable that appeared to increase the cancer risk. It has been suggested [D24], and accepted as a likely explanation [H26], that post-natal exposure of low-weight children might be responsible for such an effect. These data [H23], showing an overall 2.4 risk of childhood cancer associated with pre-natal exposure, are in agreement with the results from the Oxford series [S23] and the U.S. study [M53] and with the twin data as examined by Mole [M23]. In the view of the authors, they provide further evidence, though based on small numbers, that low-dose pre-natal irradiation may indeed increase the risk of childhood cancer.

374. MacMahon, however, in an editorial published at the same time [M55] questioned this conclusion on three different accounts. First, the small number of cases involved (31 twins with cancer), which is barely enough to give statistical significance to the excess risk of 2.4 found in the study. Second, the assumption that twins were only exposed on account of their being twins, and not also for other reasons, among which might have been the elusive factor operating in singletons (as well as, perhaps, in twins) which makes irradiated children more prone to develop cancer in childhood. Third, the possibility, mentioned in the paper, that natural cancer incidence may be lower in twins than in singleton babies. If this is true, and if twins are irradiated more often than children born single having a higher cancer risk, twins should, in fact, have a higher cancer incidence. The series may, however, have been too small to document any difference. It should also be mentioned that this latter argument may lose much of its validity because in the study by Harvey et al. [H23], mono- and di-zygotic twins were pooled and there is no a priori reason to think that these two classes may have the same natural incidence of cancer, an issue also taken up by Waight [W31]. In conclusion the new study [H23] does not seem to provide any firmer evidence in favour of a causal association between pre-natal exposure and childhood cancer.

375. Preliminary data showing a non-significantly elevated risk of malignancy in children exposed to ante-natal diagnostic radiation among 555 childhood cancer cases in Great Britain have also been reported recently [H30]. Although the relative risk of developing leukaemia or lymphoma over the first 14 years of life was 1.33 (C.I. 0.85-2.08) among exposed (as compared to non-exposed) children, it was 5.57 (C.I. 1.72-18.09) for children developing such malignancies within 2 years of age. It has already been pointed out [E8] that with the small number of cases involved when statistics are broken down, the possiblity arises of calculating a large number of relative risks and finding one "significant" purely by chance. Further analysis of this series will have to await a fuller account of the findings.

376. A summary of the relative risks of leukaemia found in studies of pre-natally exposed children since the 1964 report of UNSCEAR is given in Table 14.

C. CONCLUSIONS

1. Data from animals

377. Even though more relevant data have appeared since the 1977 UNSCEAR review, it remains difficult to provide overall conclusions on the subject of tumour induction in pre-natally irradiated animals, in comparison with irradiation of post-natal ages. It is, perhaps, appropriate to underline the difficulties hindering an overall judgement, because their discussion may help to point out the reasons why conclusions should be prudent.

378. First, there is the natural variability between species and strains with respect to the number and types of tumours arising in intact animals and the different susceptibility of various tumour types to radiation. Given this background variation, it is difficult to project information across species for the overall tumour induction or for the susceptibility to given tumour types. This objective is rendered even more difficult by the fact that information is rarely available over a large range of doses and an adequate number of animals. It should be stressed that generalizations based on only a few dose groups may be misleading, because information collected so far points to the existence of dose-induction relationships of complex forms. Just as in adult systems, curves going through a maximum, and then decreasing with increasing doses, have been documented in animals irradiated in utero. There is every reason to suspect that the placement of such maxima in respect to the dose axis and their relative height may vary with different animal species and tumour types, and to expect that, as a first approximation, wide extrapolations between species may be unwarranted.

379. Some experiments have convincingly documented differences between sexes in the susceptibility to tumours by irradiation in utero, not only in respect of tumours of the reproductive organs (or known to be dependent on the action of sexual hormones) but also in respect of neoplastic diseases, such as those of the haemolymphopoietic system, for which such dependencies are not very strong in the adult. The concomitant action of radiation on the developing reproductive and hormonal systems may to some extent be related to such differential actions. There are also indications that differential effects may be present for radiation of low- and high-LET, the latter being considerably more effective for carcinogenesis of animals in utero. Data on such matters are few and preliminary, however.

380. The notion that radiation may be consistently more carcinogenic when delivered to animals in utero than to adult animals is not easy to document on the evidence currently available: but, here again, comparisons should only be made with great circumspection. It is not a straightforward procedure to compare the results obtained from animals early in development with those obtained from animals in full maturity, because the target cell responsible for the induction of some tumour types may not yet be present in embryonic or fetal animals. Under such conditions, comparisons of induction rates may be of little value. Furthermore, for given histotypes, the target cells, even if already present, may be in various stages of differentiation, a condition which could reflect, to an unknown extent, on the final incidence of tumours produced. Finally, in view of the other radiation effects induced in developing animals (growth disturbances, malformations, killing), the dose "window" over which the carcinogenic action can be analysed is rather narrow. This adversely affects the significance of the results, because the number of animals available for analysis of tumour induction may be reduced by the concomitant presence of such effects.

381. All the above considerations apply both to external and internal irradiation. In the latter case, however, comparative estimates of tumour susceptibility between in utero and adult animals are further affected by the fact that the rate of dose delivery to the sensitive structures may be substantially different and the dose delivered to the target cells (particularly in case of tissues such as the bone) may have a biologically different meaning when delivered at various developmental stages. The structures into which the dose is absorbed, the rate of delivery and the total integration times are, in the case of internal irradiation, of overwhelming importance for meaningful assessments.

382. Under these conditions, conclusions applying to one tumour type could not easily be extended to other tumours or to all tumours. This further reinforces the notion that extrapolations in such matters should be extremely guarded and generalizations very cautious. Taking into account these numerous pitfalls, the evidence reviewed does not convey an overall impression of a significantly higher susceptibility to the induction of tumours of animals irradiated in utero. This conclusion, based on a review of all or specific tumour types, is also in accordance with the conclusions of a previous UNSCEAR review [U5]. This showed that irradiation in utero of mice produces less marked life-shortening than irradiation during post-gestational ages, or even no life-shortening at all, particularly for irradiation of the early embryo. Similarly, experience with the rat showed a doubtful effect of radiation in inducing life-shortening, and, in any case, an effect not substantially different from that produced by similar doses given soon after birth. This conclusion is also based on the assumption, exhaustively discussed and accepted [U5], that radiation-induced life shortening, at doses below the lethal range, is mainly attributable to the appearance of extra tumours.

383. Given the above reservations, it appears that the majority of cases analysed in terms of total tumour rates points to a lower, rather than a higher, susceptibility to tumour induction of the developing animals. What is most convincingly borne out from the data is that irradiation in utero produces a different spectrum of tumours than does post-natal irradiation of young or old animals. This observation is in line with the general belief that the histogenesis of the cell lines, which are the probable targets for tumour induction, proceeds at different times and rates in the developing mammal. Accordingly, one should not expect to find the same types and rates of neoplastic transformation for irradiation of the embryonic, fetal and adult stages. Furthermore, changes in tumour spectra between animals irradiated pre- and post-natally differ for different species. This is in agreement with the notions that the rate of organ and tissue formation is very dependent on the species and that each has a characteristic pattern of naturally occurring tumours.

2. Data from man

384. Data on the induction of tumours by pre-natal irradiation in man may be divided into two groups: those obtained from the survivors of the atomic bombs at Hiroshima and Nagasaki, and those derived from medical exposures. The most recent reports on the Japanese survivors have continued to show no evidence of excess cancer death among individuals irradiated in utero at the time of the explosions. Although the sample size and the number of deaths involved in this survey up to now are too small to allow firm statements, the doses received are sufficiently large, particularly in comparison with those delivered in the course of obstetric examinations, to give weight to the general conclusion. For leukaemia, in particular, a second independent report confirmed the absence of an excess risk in a sample of over 700 irradiated subjects. It is of course possible that when the atomic bomb survivors irradiated in utero will reach the older ages at which cancer normally appears, they might show higher incidence rates, as compared to non-exposed controls.

385. The Oxford Survey, the largest survey on children exposed for medical reasons while still in utero, has continued to stimulate controversial discussion. On the one hand, the group involved in the survey from its very origin have refined the analysis of the data and shown the existence of a number of factors of social and medical relevance that could affect the association between exposure in utero and proneness to develop cancer in childhood. These factors could not have been of such weight to render the association statistically non-significant.

386. On the other hand, critics of the Oxford series tend to believe that there may have been some selection of the mothers undergoing radiological examination during pregnancy. They point to differences between the social and medical conditions of children developing tumours and those not developing them, the differences being such that, for the traits

examined, the children could be considered to belong to different populations. Criticism has also been expressed of the analysis of the dose-response relationship and the way the doses (or their equivalent expressions) were defined for the purpose. Other analyses of the Oxford series data tended to disprove the causality of the association between x-ray exposure and tumour induction. According to these analyses, it is impossible to exclude that medical or other conditions leading to the selection of cases undergoing pre-natal radiology might themselves be associated with the increased occurrence of malignancy in childhood.

387. One strong argument in favour of a causal relationship between pre-natal irradiation and childhood cancer was the finding of a similar excess of leukaemia and solid tumours in children born of single or twin pregnancies, in spite of the fact that the children in the latter category had a much higher rate of radiological examinations. This argument has, however, been challenged on statistical grounds because the numerical estimates on which it had been based could be affected by such wide error limits as to render the comparisons meaningless.

388. Additional independent evidence for an association has come from the extension of a survey already reported in 1962, based on about 1.5 million children of whom more than 1300 developed cancer before the age of 20. The new study confirmed in part the previous findings for leukaemia, pointing out a 50% higher risk of developing this disease among children irradiated in utero than among non-irradiated children. In part, however, it modified previous conclusions regarding incidence of solid tumours, lowering the relative risk in the exposed population to about 15% higher than the controls from a previous value of 50%. The study also analysed and confirmed the existence of confounding variables that could potentially enhance or reduce the association between in utero irradiation and the likelihood of developing cancer. At the same time, it pointed out that correcting for such factors would be insufficient to explain the excess risk in the irradiated children. Overall, therefore, these conclusions are in reasonable agreement with those from the Oxford series. Other, smaller series have given essentially non-significant answers.

389. The experience concerning the carcinogenic action in man of irradiation in utero thus appears paradoxical in many respects. On the one hand, a prospective study on children exposed to relatively high doses from the atomic explosions has consistently failed, over the years, to reveal any increased risk [J4, K4, I3]. On the other hand, two larger, independent, retrospective studies carried out in Great Britain [S44] and in the United States [M25, M53] have consistently shown an increased risk of developing leukaemia and solid tumours in children exposed for medical reasons to much lower doses. The magnitude of the leukaemia risk is evaluated at about 50% higher than in non-exposed children, and that for solid tumours possibly somewhat less. None of the critics of these findings have denied the existence of such an apparently increased risk: they have rather been inclined to explain the finding on the ground of faulty methodology, or to deny, on various grounds, the causality of the association between exposure and malignancy.

390. The methodological defect of the Oxford series would consist, according to Totter [T6], in the use of the "excess risk". This notion is faulty, because: (a) it is based on the assumption that the frequency of x-ray examinations is similar in both the leukaemic and the non-leukaemic children; (b) it presupposes that the risk is zero for zero dose; and (c) it is based on imperfect "dose" estimates. There is also a statistical difficulty in the use of "excess risk", owing to the fact that the data points with the largest errors are given the greatest weight in the analysis. These criticisms, however, could not apply to the United States series, which uses the methodology of the maximum likelihood risk ratio as a summary statistic, and yet comes quite independently to very similar conclusions.

391. Other critics have tended to deny the causality of the association shown in the Oxford and New England series by providing evidence that the populations of children who will or will not develop malignancies are different in respect of a number of medical and socio-economic characteristics [T2]. The existence of confounding factors that could affect the association between exposure and increased risk is a well established fact, one to which the researchers themselves in the Oxford [K9] and New England [M53] series have given much attention. In both cases, the conclusion is that correcting for such factors is insufficient to disprove the significance of the association, which must therefore be accepted as sound observational evidence. Differences between the two sets of children who may or may not eventually develop a malignant disease imply that some characteristics of the mothers, of the children themselves, or of their relationship during pregnancy may have prompted a different rate of radiological examination of the two populations. These characteristics, rather than the exposure itself, could be associated with the induction of tumours. It is, of course, impossible to prove that this may not have happened, but it must also be recognized that none of the factors examined so far has been shown to be responsible for the association and it is difficult to suggest other factors for further examination [M52].

392. Other arguments in favour of a causal relationship have been proposed by Mole [M23] who examined the excess of leukaemia and cancer in children born of single or twin pregnancies and observed that, in spite of the higher rate of radiological examination of the twins, they had a similar excess of leukaemia and cancer. If valid, this would be an internally consistent proof of the causality, but Mole's calculations have been challenged [B35] on the ground that the risk in twins could actually be lower than in singletons and, in any case, the errors affecting the estimates are too large to be conclusive in either sense. Proportionality between risk and exposure might be another indirect proof of the causality, but the difficulties of assessing the doses precisely have been long recognized [S24, S26, B22, T2] and these cast doubt on the foundations themselves of the dose-response analysis.

393. In summary, therefore:

(a) The finding of a positive association between exposure in utero and subsequent development of leukaemia and cancer during childhood. in the Oxford and New England series is in contrast with the absence of any such correlation in the Japanese series;

(b) The fact that higher doses are involved in the Japanese than in the other two series is a further cause for concern, as radiobiological effects are usually expected to follow some positive function of dose. The different nature of the exposures, and the fact that the Japanese series is prospective, and the other two retrospective. do not help in resolving the difference;

(c) It is also difficult to explain why high doses given at Hiroshima and Nagasaki soon after birth show their carcinogenic potential only many years after exposure, while the relatively smaller doses involved in medical exposure in utero seem to exhaust their potential within the first 10 years after exposure;

(d) The Oxford and the New England series are at variance with data from experimental animals, which are, in the vast majority, essentially negative in terms of a higher carcinogenic risk of pre-natal exposure. Nothing can be said as to whether the change in the overall tumour spectrum, often found in experimental animals irradiated pre- or post-natally. may also apply to man, because the epidemiological series have not been followed long enough. It may, of course, be possible that, considering all malignancies that will be expressed up to the death of the human cohorts irradiated in utero and the attendant detriment, pre-natal irradiation might result in less overall detriment for the same dose than post-natal exposure;

(e) On radiobiological grounds, the constancy of the relative risk of irradiation in utero over the whole spectrum of malignancies appears to be in contrast with the well known variability of different tumour histotypes to the carcinogenic action of radiation. This observation could be due to chance, or to the short observation period of the medical irradiation series, and could still be modified with longer follow-up, but it remains, at the present state of knowledge, unexplained;

(f) The constancy of the relative risk also implies proportionality between natural and radiation-induced malignancies over all developing tissues (at least up to the present stage of follow-up) which is by no means a common experience for irradiation of adult systems;

(g) Similarly implausible on embryological and radiobiological grounds is the observation of a higher relative risk of leukaemia for exposure during the first trimester (than during the last two) when it is known that adult haemopoiesis has not yet begun and target cells to be transformed into leukaemic cells have not presumably differentiated.

394. It is, in the last analysis, the joint consideration of the findings described, and their evaluation in the light of all the above-mentioned inconsistencies, that may determine acceptance or rejection of the causality of the relationship, because data, to be credible, should fit into an overall logical framework accounting for all of them, and not for only a few. It is interesting to note, in this respect, that the authors involved in the Oxford series favour the causal nature of the association, while those involved in the New England series reject such a notion. In the view of UNSCEAR, the important consideration is the existence of the association. For the Committee's own purposes to neglect the matter until (and if) it is given some plausible explanation would be unacceptable because, if the association exists, it could, to some extent, affect the estimates of radiation burden in man. Under the circumstances, the only possible course of action is to recognize the association and to assume its causal nature, keeping in mind, however, that its true nature remains unresolved because the data do not fit into an overall logical picture of radiation carcinogenesis as it is currently understood. It is to be hoped that further, independent data will help to resolve the existing discrepancies, although it appears unlikely that direct evidence will ever be produced for lack of suitable material to study. In choosing this course of action, UNSCEAR wishes to stress that acceptance of the causality of the association is assumed simply on account of prudence for any relevant practical consequences. On purely scientific grounds, in fact, the issue should either be dismissed for lack of scientific coherence or should be left undecided, pending further new evidence. UNSCEAR, although its functions are only those of a body assessing data on their scientific merit, is not insensitive to the practical consequences of its deliberations. Thus, it believes that not to include the potential harm of exposures in utero (whatever it may be) in the total balance of radiation risks would unduly weight scientific orthodoxy against the practical needs of safety. The Committee will, of course, be ready to modify this position on the strength of any new scientific evidence that might be forthcoming.

VIII. RISK ESTIMATES

A. GENERAL

395. Quantitative risk estimates for radiation damage after exposure in utero are of considerable importance for their possible practical implications. An early expression of the absolute amount of risk likely to occur under these circumstances is to be found in the 1969 UNSCEAR report [U1]. This stated that, in respect of severe mental retardation associated with a reduced circumference of the skull, an estimate of the risk coefficient derived from observations in Japan, with high acute doses in excess of 0.5 Gy, might be set at about $10^{-1}\,Gy^{-1}$, averaged over the gestation period. The report stressed the uncertainties involved in extrapolating such a value to smaller doses, in the absence of suitable human data and with insufficient knowledge of the underlying mechanisms. The 1977

UNSCEAR report [U2] endorsed this provisional value, again underlining the limitations under which it had been derived and the difficulties involved in extrapolating it to other exposure conditions. The report also pointed out that a survey of the available human experience, with doses in the region of 0.01-0.2 Gy, had given negative or inconclusive answers. They could only be of indirect value in excluding the possibility that, at such doses, the human embryo might be 10 times more sensitive than implied by the incidence of malformations at higher doses.

396. The 1977 report [U2] was criticized in a paper by Mole [M36] as being an inaccurate account of facts which could convey the impression that radiation exposure of the human embryo in its early stages could be positively harmful and thus might engender apprehension about the relevant risk. The paper in question is a complex one. It comprises a text aimed at setting risk in perspective (particularly with respect to the normal risk of handicaps at the end of a normal pregnancy), in view of a reconsideration of the so-called 10-day rule, and an appendix setting out in detail the criticisms of the UNSCEAR report and the arguments on which the main conclusions rest.

397. While recognizing that the discussion of experimental findings could in each instance be extremely detailed, UNSCEAR believes that this should not be the specific purpose of its assessments, which aim at overall evaluations of specific fields. It believes that the assessment of developmental effects of irradiation in utero, on the basis of the evidence available in 1977 [U2], should not be modified on account of the criticisms raised. It also believes, however, that the most recent findings in this field, and the widespread attention they have commanded, have made the field a particularly worthy subject for further study and review.

398. In other papers [M9, M10], Mole discussed, and rejected, the notion that the very early human conceptus (0-14 days) is an especially radiosensitive organism. This notion, he found, was derived from too-facile generalizations based on laboratory animal experiments, without due consideration for the specificity of human development. First, he examined the most recent evidence on small head size and mental retardation from Hiroshima and Nagasaki [O3] to show that the distribution of cases, as a function of the week of gestation at the time of exposure, is incompatible with the hypothesis that the earliest stages of pregnancy are the most sensitive in regard to impairment of mental function. This should, of course, be expected on the ground that embryo killing (either pre- or post-implantation) and malformations, and not mental retardation, are the types of damage expressed by irradiation at the early stages.

399. Mole then underlined [M9, M10] the good correlation, originally pointed out by Dobbing and Sands [D12], between the period of rapid proliferation of neuroblasts in the developing human CNS between about 10 and 18 weeks of pregnancy (8-16 weeks after fertilization) and the clustering of mental retardation cases at around the same time in the Japanese experience. This observation (which had been previously overlooked by workers in radiation biology and by the 1977 UNSCEAR report) is indeed very valuable because it correlates precisely cellular events in the human brain with radiation-induced functional damage. It is, however, in line with the general notion that the sensitivity of a given structure is highest at the time of its maximum growth, an observation repeatedly documented for many structures in experimental animals and to which the human experience would be expected to conform.

400. Mole further discussed [M9, M10] the significance of malnutrition in connection with the Japanese experience, pointing out that food shortage before, and particularly after, the bombing might have been responsible for an unusually high control level of mental retardation and might, if so, have led to an over-estimate of the effect of irradiation (under the hypothesis of a combined action). Mole also discussed points such as: the effect of excluding 2 cases of Down's syndrome in the irradiated subjects and 1 case of encephalitis in infancy in the control subjects; the difficulties with the definition of microcephaly as diagnosed at Hiroshima and Nagasaki; the lack of correlation between small head size and mental retardation; the effect of perinatal mortality; and the lack of other data on morphological abnormalities in the Japanese series. The author also reviewed other series, to show the relative infrequency of major developmental malformations as commonly understood.

401. In that part of the paper [M9] which refers to observations on laboratory animals, Mole stressed that species intercomparison should only be carried out with due regard for the timing and duration of developmental events. This conclusion is quite acceptable when actual times or rates of induction are discussed (which was never the case in the 1977 UNSCEAR report [U2]) but not when projections across species are so general as simply to point out the good correlation, found in various species, between stages of organogenesis of structures and their sensitivity to irradiation.

402. Finally, Mole discussed [M9] the mechanisms of morphological and functional damage following irradiation in utero, pointing out the distinction that should be made between "malformations" resulting from failure of embryonic organization and having clear teratogenic connotations and "maldevelopment" resulting from cell depletion distributed randomly throughout an irradiated tissue. Mental retardation would belong to this second class of damage. Difficulties in the definition of malformations and growth disturbances, and the frequent association of various types of damage, were discussed at length in the 1977 UNSCEAR report [U2]. In the light of the above definitions, Mole believes there is no evidence in the human species to show that radiation is an efficient teratogen.

403. When teratogenic effects are described, a distinction is often made between malformations and growth disturbances, even though it is recognized that

the two effects are often associated. In a paper by Spiers [S15], the hypothesis is developed that growth retardation and teratogenic effects may not be independent end-points of common causes. Evidence for this is drawn from epidemiological observations on some human congenital malformations in twins; from multiple as opposed to single malformations; and from the association between malformations and growth retardations in patients with Down's syndrome and in children from normal and diabetic mothers. On the basis of analyses carried out on such data, it is argued that growth retardation may represent a state of increased susceptibility to congenital malformations in the sense that they will be more likely to occur when the overall growth rate of the fetus is grossly disturbed. A more recent paper by Tchobroutsky et al. [T13] has pointed to a high correlation between fetal defects and early growth delay observed by ultrasound, thus confirming that growth retardation may constitute a state of increased susceptibility to the occurrence of congenital malformations.

404. In another paper [M21], Mole discussed the latest evidence from Japan, emphasizing the good agreement between embryological and radiological findings in man and the absence of any record of maldevelopment other than mental retardation. This paper also contained a valuable review of case reports in the literature, pointing to mental retardation as the most significant category of damage in the human species. As to experimental observations, the paper accepted that pre-implantation irradiation of the conceptus leads to a dose-dependent reduction of surviving normal individuals, while irradiation during the period of major organogenesis causes a great variety of visible malformations. In pointing out the differences between findings in man (where true malformations are uncommon) and in other animals (where teratological damage is prevalent), the paper suggests that a slower rate of cell division of the human embryo might be the reason for such differences.

405. The fact that in man growth disturbances, small head size and mental retardation are the predominant effects of acute exposure during pregnancy was also noted by Brent [B25-B27]. This is at variance with data in experimental animals [U2] in which malformations of different organs and structures are a common finding after comparable doses. According to Brent, there may be various explanations for such differences. First, development of the CNS in man proceeds throughout gestation and well into the neonatal period [D12, H14], while other organ systems develop over a much shorter time. For example, in man organogenesis may last from the 2nd to the 8th week of gestation, corresponding to about 5% of the whole pregnancy, while in the rat it may last from day 9 to 13, corresponding to 20% of pregnancy [P11]. Secondly, the manner of exposure is usually different in animal and human populations. In the first case, exposure is often aimed at inducing specific malformation by selective irradiation at certain times, while in man exposure has usually occurred at random and has therefore been relatively more likely during the fetal stages when sensitivity of the developing brain remains high.

406. In 1977, the International Commission on Radiological Protection (ICRP) [I4], in the context of a general attempt to develop an index of harm applying to a working population for radiation protection purposes, provided some estimates of risk during various stages of pregnancy and for various types of effects. In summary, the risk of lethality (before implantation or in utero) for irradiation during the pre-implantation stages was estimated, by extrapolation from data in rodents, to be of the order of 10^{-3} a^{-1} for exposure of a female working population at the rate of 1 Sv a^{-1}, weighted over the 8 days pre-implantation during which this risk was assumed to operate. (This rate of exposure was taken simply for comparative purposes.) For all malformations occurring after exposure at the same rate during the period of major organogenesis (assumed to last for 17 weeks during pregnancy) the risk was set at 10^{-3} a^{-1} Sv^{-1}. The risk of inducing a fatal childhood malignancy was set at 2.3 10^{-2} Sv^{-1}. On the assumption that the risk of fatal malignancy in females (allowing for breast cancer) is 1.5 10^{-2} Sv^{-1}, that the fetus is exposed for an average period of 7 months during which the mother remains at work at the rate of 1 Sv a^{-1}, and that the average number of births per year per woman is 0.065, it was further calculated that the average risk of inducing a fatal childhood malignancy would be about 6% of that in the adult member of a female working population.

407. In 1979, Mole [M36] addressed the problem of quantitative risk estimates and produced the following figures. For mortality after exposure to x or gamma irradiation in the 1-4 months embryo or fetus, 0-1 additional cases per 1000 births after a tissue dose of 0.05 Gy low-LET radiation: for mental retardation in adolescence, 0-25 additional cases per 1000 live-born after 0.5 Gy tissue dose (0-1 case after 0.05 Gy); and for malignant diseases before the 10th birthday, about 50 excess cases per 1000 of all live births after 0.5 Gy tissue dose (about 5 cases after 0.05 Gy). Such estimates, derived from evidence concerning brief exposures, would not be expected to be strongly influenced by dose protraction because most of the risk is attributable to cancer production. Scaled down to 0.01 Gy of low-LET radiation on a linear non-threshold hypothesis, the overall risk would lie in the range of zero to 10^{-3} cases, which should be compared with the natural probability of occurrence of serious handicaps in average pregnancies, which was estimated to be upwards of 3 10^{-2}.

408. Brent [B25, B27] discussed the risk estimates from irradiation in early life. He stated that, since, contrary to most other teratogens or embryopathic agents, radiation has an effect on the developing embryo which is direct and not mediated through maternal metabolism and placental transfer, one may extrapolate the results of irradiation experiments with pregnant mammals to the human more readily than with any other teratogen. However, in view of the numerous differences pointed out in the developmental pattern and in the response of man and other mammals, UNSCEAR believes that great caution should be exercised in such extrapolations, especially

for the more quantitative projections. Moreover, risk estimates ultimately to be applied to humans for practical purposes should essentially be derived from human experience, carefully adapted to the special situation to which exposure applies.

409. In order to estimate risk, Brent summarized, in tables, the effects of acute exposures at 1 Gy and at 0.1 Gy or less, on various developmental stages of the rat and mouse (displayed in relation to corresponding human gestation periods). He then extrapolated from them the minimum doses to produce lethality, malformations, growth retardation and other effects in the human embryo, as in Table 15. Brent's conclusion from these data is that the hazard of exposure in the range of diagnostic roentgenology is extremely low when compared with spontaneous risks in human pregnancy. Tubiana [T4] estimated that a dose of 0.02 Gy to a fetus could induce, at the most, the risk of 1 case of cancer in 2000 children.

410. Kameyama [K22] addressed the problem of risk to the human embryo and fetus by simple extrapolation to humans from observations on the lowest effective doses in experiments with rats and mice, warning, however, about the danger of such straightforward projections. In a general review of the field, he estimated the lowest doses for non-stochastic effects in the mammalian embryo and fetus to be as follows (figures given in Gy to the relevant tissues): for resorption of pre-implantation embryo, 0.05; for acute cytological changes, 0.05-0.1; for minor skeletal anomalies, 0.05; for malformations, 0.15-0.20; for histogenetic and functional disorders of the CNS, 0.20-0.25; and for impaired fertility, 0.20-0.25. From these data, Kameyama concluded that the risk of non-stochastic effects of diagnostic irradiation of children in utero, for doses which are normally less than 0.01 Gy and up to 0.02 Gy as a maximum, would be extremely low, and probably negligible, given the appropriate control measures. It should be noted that carcinogenic and mutagenic effects are specifically excluded from these estimates, although these effects, according to other evaluations, constitute the major portion of the overall risk.

411. Russell [R8] discussed the problem of critical periods in the course of development. Although her contribution has mainly methodological value, it does contain observations of very general importance, particularly regarding the role of sensitive stages in the estimate of the attending risks. It points out, for example, that to consider the embryo as a whole, without defining the sensitivity of its cell subpopulations in the course of time, is a simplification that may produce a loss of sensitivity in the detection of risks, i.e., in an under-estimation of it. Conversely, extrapolation of the risk from single short-term exposures, carried out during high susceptibility stages, to protracted exposures, may lead to over-estimation of the risk because a given effect which can readily be induced with an optimum dose at a well defined stage of development may not be visible at all at other stages, even those occurring in close proximity to the critical periods.

B. EFFECTS AND PERIODS OF MAXIMUM SENSITIVITY

412. Before attempting any quantitative estimate, it is necessary to examine the nature of the risk under discussion and the time periods in human development over which such risk might apply. The effects that have been identified in the preceding discussion as relevant to human irradiation are reviewed below. For exposure during the pre-implantation phases, animal data (particularly in rodents) have documented pre-implantation death. As discussed in the 1977 UNSCEAR report [U2] this is more readily apparent in polytocous animals and may appear as death of single individuals (inferred from a dose-related decrease of the average number of implants) or as death of whole litters (documented by a dose-related increase of sterile matings). There are no data that might suggest the presence of such an effect in man. This is probably due to the fact that it is difficult to observe. Pre-implantation loss would actually be likely to result only in a missed menstruation, an event of such minor clinical consequence that it is hardly necessary to account for it. However, there is every reason to expect that a sufficiently high dose of radiation received by a pre-implanted human embryo would entail a definite probability of death: the question is, what magnitude should be assigned to this risk? In vivo data from the mouse, which were discussed in the 1977 report [U2] (and which have not been contradicted by the latest evidence on embryos irradiated and cultured in vitro, discussed in chapter II) point to a net increment of pre-implantation embryonic loss coefficient of the order of 1 Gy^{-1}. (For the purpose of the present study, the exposure unit R will be taken to give an absorbed dose of 0.01 Gy, a procedure that is not likely to underestimate the risk.) Evidence in rodents reviewed in the 1977 report [U2] is not incompatible with non-threshold dose relationships for irradiation during the pre-implantation stages. The same could be said for the in vitro data reviewed in chapter II. As to the length of the period over which such a risk could remain in effect, it might extend as a maximum over the first two weeks from fertilization.

413. In man, the phase of major organogenesis may be taken to last approximately from the 2nd to the 8th week after fertilization. Earlier in the present study, attention was called to the fact that, contrary to what happens for other mammalian species (e.g., rodents), where the relative duration of organogenesis over the whole period of pregnancy is longer, there is little or no evidence in the human that malformations in the teratological sense are to be seen. This may mean that: (a) the observations available for irradiation during organogenesis are too few; or (b) that exposures were too low; or (c) that grossly malformed fetuses are eliminated in the course of further development; or (d) that the human species behaves differently from other mammalian species in the sense that radiation during organogenesis may not result in gross teratological damage. The first of these alternatives is certainly true: the shortage of human data concerning irradiation of major organogenesis stages was pointed out in chapter III. The probability may not be excluded that a wider data base, or higher doses,

might have provided evidence in the positive sense. The other alternative, that grossly malformed fetuses are lost, is actually a well ascertained fact: as pointed out in chapter I, the incidence of malformed embryos decreases as a function of their increasing age. The phenomenon is to be seen as a result of a progressive elimination of embryos and fetuses carrying malformations that are too severe to be compatible with further development in utero or extra-uterine life. As to the human species being an exception to the general behaviour of other mammalian species, in the sense that it does not respond to irradiation during the phases of major organogenesis with induction of teratological defects, this alternative is hardly credible. As pointed out before, the lack of teratological effects, compared with maldevelopment of the CNS, may be justified on different grounds: for example, the much longer duration of CNS development in man over the time for organogenesis of other body structures.

414. It may be asked whether, for the purpose of risk estimation, the lack of data on teratological damage in man (compared with the relatively more common occurrence of CNS lesions) is sufficient reason to discard the possibility that such damage might occur at all. UNSCEAR believes that it is not, and will assume accordingly that such damage might occur during the 3rd to 8th week post-conception. Since the number of body structures that could be damaged is roughly similar for all mammals, irrespective of their body size, one could assume, if one considers together malformations of all types, that the ratio of malformed to normal fetuses per unit dose seen in animals may be taken as an expression of the risk of malformation induction per unit dose over the period in question for man. Therefore, for UNSCEAR's purposes, it will be assumed that the risk of an absolute increase of malformed fetuses of the order of $5\ 10^{-1}\ Gy^{-1}$ (see paragraph 95) in animals might apply to the human species as well, over the period from 2 to 8 weeks post-conception. This must be interpreted as an upper value. (Here again the exposure unit R is taken to give an absorbed dose of 0.01 Gy on the average over all embryonic structures.) It is actually possible that the most severe of these conditions may never be seen in man, because they are aborted in early stages of development. It must be emphasized that using data derived from experimental animals on the assumption of linearity, and projecting these data to man, is likely to result in an upper estimate of risk, not only because radiation-induced teratological defects are not seen at low doses in humans, but also because the existence of a threshold may not be excluded in any species.

415. Data in animals have shown [U2] that as development of the embryo proceeds, the dose needed to kill the embryo becomes greater and the form of the dose-response relationships becomes increasingly sigmoid. For the purpose of UNSCEAR's estimates, doses resulting in death of the embryo post-implantation are too high to be considered in the present context. They would not be relevant to the exposure of the population, but only to exposure of pregnant women under accidental conditions. The evaluation of such cases is clearly beyond the scope of the present exercise, which aims at providing estimates of general validity for the exposure of the whole population, or large population groups, under controlled exposures. Death of the embryo in utero during embryonic or fetal stages (or death during post-natal life as a consequence of exposure in utero), therefore, is not considered further here.

416. The number of children classified as severely mentally retarded is increased by exposure in utero. From the data discussed in III. B, it appears reasonable to assume that the risk coefficient for low-LET radiation over the period 8-15 weeks is $4\ 10^{-1}\ Gy^{-1}$ and over the period 16-25 weeks, $10^{-1}\ Gy^{-1}$. Before 8 weeks the risk is apparently zero and after 26 weeks the risk is very low.

417. Growth disturbances and other effects have also been shown to occur for exposure during the fetal period. Quite aside from the clinical significance of a reduction in body size in the absence of other clinical signs (which is indeed doubtful), it is difficult to express growth disturbances per unit dose and it is therefore impossible to provide a value of the risk for such graded effects. As for those effects on the developing gonads, which result in reduction of the reproductive capacity, it has been shown that distinctly sigmoid relationships prevail for these effects, so that it may be assumed that they may not be observed at doses of one tenth of a Gy or less. Accordingly, no attempt will be made to estimate the risk for these effects.

418. The last type of effects to be discussed is the induction of leukaemia and other solid tumours. Reasons have been given in chapter VII for assuming the existence of a causal relationship between pre-natal irradiation and induction of malignancy, in spite of the fact that, on purely scientific grounds, the supporting information is not entirely convincing. At face value, data show that the risk of developing a malignancy after irradiation in utero at doses of the order of 0.01 Gy is fully expressed within the first 10 years of life and is increased by about 50% over the normal rate. The risk may be estimated, according to a previous UNSCEAR analysis [U2] to be of the order of $2\ 10^{-2}\ Sv^{-1}$. Some evidence indicates [C13, M36] that the risk during the first trimester might be substantially higher than that for the last two trimesters. The relevant data appear unconvincing, however; constancy of the risk throughout pregnancy is therefore assumed in the following discussion.

C. RISK ESTIMATES

419. The risk estimates given in the preceding section relate to effects of different nature and severity. Therefore, they cannot be easily compared or aggregated. They should be viewed in the light of a number of qualifications, as follows:

(a) The risk estimates have been specified as if the dose relationships for the relevant effects were linear, although, as previously discussed, many dose-effect relationships for the most complex functional non-stochastic effects have actually been shown to be curvilinear (concave upwards). The estimates are therefore likely to be in excess

by some unknown factor, particularly at the very low doses.

(b) The risk estimates have been derived by using data on acute irradiation at high, rather than low, dose rates and apply therefore to this irradiation condition. Basic radiobiological considerations suggest that projection of these estimates to low-dose rate low-LET irradiation could cause these estimates to be lowered by some factor, at least for those effects that are characterized by curvilinear dose-response relationships. It has, however, been argued [M36] that no modification of the risk would occur in the case of tumour induction, which is regarded as a process initiated in a single cell. If this were true, the overall reduction of incidence due to low-dose rate would thus depend on the relative importance of cancer induction and other harmful conditions.

(c) Overall, for small doses, the detriment calculated from the risk estimates given in the previous section appears rather small, in comparison with the natural prevalence of malformations at birth which has been taken in chapter I section C to be of the order of $6\ 10^{-2}$. For example, a dose to the conceptus of 0.01 Gy delivered over the whole pregnancy would add a probability of health effects (mortality and induction of malformations, severe mental retardation, leukaemia and other solid tumours) in the liveborn of less than 0.002 to the natural probability of 0.06 of a new-born child being seriously handicapped. In addition, it should be kept in mind that when radiation is delivered acutely, not all the above risks will operate jointly; on the other hand, for radiation delivered chronically, for reasons developed in point (b) above, the risk would be expected to be lower.

IX. CONCLUSIONS

420. As a follow-up to its 1977 study [U2], UNSCEAR has undertaken a revision of data pertaining to the effects and risks of pre-natal irradiation. The revision has taken into account data in human embryology, particularly of the CNS, which were either unavailable or not sufficiently well established at the time of the previous study; new information derived from experimental animal systems; and re-evaluated dosimetric and medical data on children exposed before birth during the explosions at Hiroshima and Nagasaki. All this called for a new assessment of a field that has attracted much attention over the last few years. In addition to covering such effects as tumour induction by pre-natal irradiation, this new revision aims to provide a consideration in depth of the human data and attempts to quantify the risk to man by irradiation in utero.

421. Data derived from human material have been helpful in mapping out, with increasing precision, developmental events that are of importance in the context of radiobiology. Research on morphological embryology is yielding an accurate description of human developmental stages at the same time that new techiques of observation in vivo are establishing a good correlation between descriptive morphology and body size in embryos and fetuses. The histogenesis and development of certain structures, such as the CNS, which are particularly susceptible to radiation damage at pre-natal stages, are also becoming better known at the biochemical level. Through biochemical, histological and cell kinetic analysis, the main events in the development of the brain structures are being followed; and the overall process of brain maturation is emerging as a long sequence of phenomena lasting well into the post-natal stages of development.

422. In primates, new techniques of cell labelling have allowed the reconstruction, to a remarkable degree of precision, of the kinetics leading to the formation of the cerebral cortex. It has been established that cell division, cell migration and cell maturation in a tightly arranged sequence of time and events are involved in the formation of the brain cortex. The time of most active division of undifferentiated neuronal cells is the most sensitive to the radiation insult in man. This is in good agreement with what had previously been ascertained in experimental animals. Radiation-induced disturbances of cell division, migration and maturation invariably result, through various mechanisms, in a loss of neuronal function. This loss may not be repaired by other neuronal cells, which are unable to divide, or by glial cells, which have no neuronal function.

423. After irradiation, congenital anomalies of other body structures, besides those of the CNS, are uncommon in man, but very common in experimental animals. The apparent discrepancies have been discussed, and reasons and explanations given. The relevant findings must be taken as warnings against any indiscriminate attempt to project to man results obtained in animals. Any such projection must take into account the embryological peculiarities of each species. Extrapolations, particularly of the more quantitative findings, are, in principle, unjustified.

424. Congenital malformations in the human species may be classified, according to their etiology, into those of simple genic origin (about 6% of all malformations scored at birth), those having a complex multifactorial origin (about 50%), those due to chromosomal defects (about 5%) and those known to be associated with a variety of environmental factors (about 6%). For about one-third of all malformations seen at birth, therefore, no apparent cause can at present be identified. Children malformed at birth are the survivors among a greater number of malformed embryos and fetuses who have died at some stage of intrauterine development. If malformations are scored in grown-up children, the incidence is, again, higher than at birth. Total incidence figures are highly dependent on the method of scoring; the size of sample; diagnostic criteria; ethnic and genetic background; age and parity of the parents; and seasonal, geographical and socio-economic conditions. The same is true for the various classes of malformations. As a convenient average for its assessments, UNSCEAR has adopted a

natural figure of about 6% of newborn children being affected by anomalies that may seriously reflect on their viability and physical well being.

425. No new data have appeared on the effects of irradiation in man during the pre-implantation stages. There have been, however, many new findings on in vitro irradiation and culture of rodent embryos. A generally decreasing sensitivity to killing of pre-implanted embryos, as a function of time and complexity of development, has been confirmed by these new data. There are also ample oscillations of sensitivity in relation to the various phases of the cell cycle during the earliest segmentation divisions. From the whole of these data, killing of single embryos, or of entire litters in polytocous animals, emerges as the most important effect of pre-implantation irradiation. For rodent irradiation in vitro and in vivo, doses of the order of 0.1 Gy or less have been reported to have a significant effect on embryonic and fetal mortality.

426. Recent data on the irradiation of experimental animals during the stages of major organogenesis have added significant details to previous conclusions of UNSCEAR [U2], but have not changed them drastically. It remains true that the effects resulting from irradiation of experimental animals during this phase are mostly the teratological ones, accompanied by growth disturbances of the various body structures or of the whole body. There is a pronounced time dependence for each class of malformation, because the period of maximum sensitivity of any one structure coincides with the time of its most active proliferation and differentiation. For each specific type of malformation, particularly those of the skeleton and of the CNS for which new data have been gathered, sigmoid dose-response relationships are usually found. A wealth of data on the pathogenesis of malformations in the CNS has been of help in understanding how the radiation damage to the developing neuronal cells might be translated into functional CNS damage, through the disturbance of division, migration and intercellular connection of the irradiated neurons.

427. Sensitivity to radiation damage in the human CNS starts at the borderline between the end of organogenesis (8 weeks after conception) and proceeds well into the conventional fetal stages, up to 25 weeks. An important step forward in the analysis of this effect and the attendant risk has been made possible through a review of dosimetric and clinical data on children irradiated in utero during the atomic explosions at Hiroshima and Nagasaki. It has been confirmed that some of these children have developed clinically severe mental retardation and the incidence of this condition has been analysed as a function of post-conceptional time at irradiation and as a function of the dose received by the embryos, according to the most recently available dosimetric evaluations. Mental handicap is not seen in children irradiated up to 8 weeks from conception, is at a maximum between 8 and 15 weeks when neuronal proliferation is most active and decreases to lower values between 16 and 25 weeks, when glial cell proliferation and neuronal synaptogenesis occur. The number of children classified as severely mentally retarded is increased by radiation exposure in utero. The excess number of mentally retarded in the dose range (0.04-2 + Gy) is apparently linear with dose at 8-15 weeks, with an average risk coefficient of 4 10^{-1} Gy^{-1}; it is, on the other hand, concave upwards at 16-25 weeks with a coefficient of the order of 10^{-1} Gy^{-1}. There is an indication that, in addition to these extreme mental handicaps, other less prominent functional brain deficits might be present in children irradiated in utero. While not all aspects of these findings may be explained on the basis of present radiobiological knowledge, their significance is thought to be high, particularly in respect of the quantitation of the attendant risk.

428. For irradiation of animals during the fetal stages, a variety of effects have been described, but those on the developing gonads have been particularly well documented, both at the anatomical level and also in respect of the ensuing loss of reproductive capacity when animals irradiated in utero reach the fertile age. Although the form of the dose-effect relationships for loss of germ cells may (at least under some conditions) appear to be exponential, pronounced sigmoid relationships with dose appear to prevail for impairment of reproductive capacity, with a fair degree of inter-strain and inter-species variability. Doses of a few tenths of a gray as a minimum, are necessary to elicit observable fertility changes in various animal species. Other effects induced by fetal irradiation have been described for the haemopoietic system, the liver and the kidney. Such effects all occur at fairly high radiation doses.

429. The effects of internal irradiation of the mammalian conceptus are greatly influenced by the nuclides tested, their chemical form, the route and schedule of administration, and the kinetics of transfer and metabolism of the radioactive substances from the mother to the fetus through the placenta. Information on all these aspects, for all nuclides, is far from complete and only a few nuclides appear to have received some attention. Tritium has been investigated in relation to its different chemical forms, its metabolism, the type of effects induced as a function of dose, and its relative biological effectiveness by comparison with external gamma exposure. Radioiodine has been studied in respect of its kinetic behaviour following acute or protracted exposures, through a comparison of the uptake in the mothers' or fetus' thyroid. Plutonium distribution and incorporation in various organs of the mother and the fetus have also been studied in three different mammalian species in an attempt to compare the findings among species. The available evidence does not show a preferential tendency of actinides to concentrate in the embryo and fetus. There appears to be a need to enlarge the data base for the nuclides of importance, particularly in regard to their uptake, distribution and effects over an adequate range of concentrations and tissue doses.

430. Radiation effects in the mammalian conceptus may be modified to different degrees by a number of factors. Among these factors, the type and energy of radiation have been investigated. Relative biological

effectiveness (RBE) values in the region of about 5 for fast neutrons and helium ions (as compared with gamma radiation) have been reported, with a clear indication that the actual values could vary according to the types of effects scored and the dose level. An increase of RBE with decreasing radiation dose has not been convincingly shown, but may be expected from the shape of the dose-response relationships reported so far. Information on the interplay of LET, the oxygen effect, and dose-rate or fractionation has not been found to be very abundant for effects in utero, although there is no reason to suspect that the developing conceptus may behave differently from adult mammalian systems in respect of these major radiobiological parameters.

431. In the field of combined actions, ionizing radiation has been found, in isolated instances, to behave synergistically with some chemical agents, ultrasound or high-frequency radiation. More thorough and systematic studies would be required, however, to substantiate these reports over a wider range of doses and effects. Radioprotective and radiosensitizing drugs have also been tested in combination with radiation; the results indicate that the developing tissues are roughly similar to adult ones in their response to these substances.

432. UNSCEAR has analysed and assessed a large body of literature on tumour induction following in utero irradiation of experimental animals. It has identified a number of weak points in these contributions which make it difficult to draw overall conclusions, particularly for a comparison of the carcinogenic action of radiation in pre- as opposed to post-natal stages. The species, strain and sex variability, the lack of extended dose-response analyses, the different stages of organ histogenesis and differentiation, the variable types and dosages of radiation, as well as objective methodological and technical difficulties, may affect any such straightforward comparison. However, there is no good evidence of a significantly higher susceptibility to tumour induction of pre-natal animals. On the contrary, the data point to a lower susceptibility upon irradiation in utero. In any case, the most striking effects are on the spectrum of the types of tumours developed after irradiation of pre- or post-natal ages, rather than on the incidence rate of all tumours.

433. Data on tumour induction by pre-natal irradiation in man come from two different major sources. On the one hand, the survivors of the atomic explosions at Hiroshima and Nagasaki, who were exposed while still in utero, have continued to show, upon successive reviews, no evidence of excess cancer. On the other hand, two much larger retrospective surveys in children exposed in utero on account of medical indications have consistently shown an excess of tumour and leukaemia cases over the first 10 years of post-natal life, which may be estimated to be roughly 50% above the natural incidence for the doses involved in those studies. A number of social and medical factors have been identified that may affect the association between irradiation in utero and proneness to develop cancer in childhood. However, these factors are not of sufficient weight to explain the association entirely. Other reasons have been discussed which render the results of the different series difficult to reconcile.

434. The authors involved in the analysis of the two retrospective surveys described disagree about the causal association between irradiation in utero and the likelihood of developing leukaemia and cancer. UNSCEAR believes that the important consideration in these matters is the existence of the association, which has been firmly established. As to the causal relationship, to deny it on the ground that such positive evidence does not agree with the experience in Japan or the data in animals would mean putting undue weight on scientific considerations over the practical need of allowing for any possible risk. Therefore UNSCEAR accepts, provisionally, the causal nature of the association, for practical purposes, but emphasizes that this is simply on account of prudence and not on any scientific grounds. UNSCEAR will follow these matters and be ready to modify its position on the strength of any new evidence.

435. UNSCEAR has attempted to work out quantitative risk estimates for a number of radiation-induced effects in utero (mortality and induction of malformations, mental retardation, tumours and leukaemia) and to attribute the risk to the period of pregnancy over which it applies. Under a number of qualifying assumptions, it is possible to conclude that, for the small doses likely to be encountered in practice, the overall risk is relatively small (no more than 0.002 for the liveborn at 0.01 Gy) in relation to the natural incidence of malformations in non-irradiated individuals, which is of the order of 0.06 in the human species.

X. RESEARCH NEEDS

436. At various points in the preceding text attention has specifically been drawn to areas where there is a lack or substantial limitation of information that might enable full understanding and assessment of the data in man on which risk estimates should be based. In other cases such limitations have been implied in discussing the difficulties of deriving sufficiently firm generalizations. It appears useful to summarize such research needs, in the hope that they might be considered when planning future work.

437. Any occasion to collect information directly from the human species should of course be pursued with the highest priority. This applies first of all to observations on radiation effects on embryos or fetuses irradiated in utero, although it appears that the material available for further studies is rather limited. In this respect, re-examination and updating at suitable intervals of children exposed for medical reasons would still be useful to search for possible long-term effects of radiation. Similarly useful and very important is the analysis of mental and physical development, school performance and other functional tests on the survivors

of the atomic explosions irradiated in utero, in order to complete the picture with the more subtle and less conspicuous effects of irradiation that may have affected these subjects.

438. The need for more detailed quantitative information applies equally to normal human development, as a necessary background for the study of radiation-induced effects. There is a need for a fine description and timing of the landmarks of human development during the embryonic and fetal stages, the variability in the occurrence of such landmarks as a function of time, and of ethnic and other characteristics. Attention and support to institutions whose scope is to describe such developmental events is important, because this knowledge is fundamental to many branches of the medical and biological research.

439. In view of the limited amount of information that may be obtained directly on human subjects, the use of models of radiation effects in utero will continue to be of paramount importance for the understanding of mechanisms and the projection of effects and risks. Although all models may be useful in different respects, depending on the answers required from them, it is to be expected that research on small mammals (rodents in particular) may be more suitable for the study of mechanisms, while research on higher mammals (primates) may be more interesting for extrapolation of the effects to man. In both cases, it is the consequences of small doses (below about 0.1 Gy of low-LET and correspondingly lower doses of high-LET radiation) that should be particularly studied. This implies designing and testing of techniques which may be sensitive to such small doses, while retaining significance and value for predictive purposes.

440. In very general terms, considering the large sample size needed, models in rodents are probably more useful for the analysis of dose- and time-effect relationships, the study of repair effects, the analysis of variability inter- and intra-species, the analysis of combined modalities of treatment, the comparative effects of radiation of different types and energy and different patterns of radiation exposure. Comparative studies on different species should also be useful in the analysis of the time sequence of various radiation-induced phenomena as they correlate with the occurrence and the duration of developmental events in each particular species.

441. It should be realized that, particularly in the case of the development of nervous system and radiation effects thereon, the size of the structures to be analysed and the duration of the phenomena involved make it preferable to use primate species for more meaningful extrapolations to man. The same may be said for the study of somatometric growth of the body and brain and for the analysis of higher nervous functions in such animals. There is also a need to correlate in these animals morphological with functional effects by making use of modern techniques of behavioural analysis. However, financial and other limitations involved in this type of research should be recognized.

442. It is also important to realize that it is no longer possible to view the brain as a single organ, but to analyse the developmental events and radiation-induced changes systematically in the different structures of the brain, to apply the newest neurobiological and neurochemical techniques, to move from the study of effects such as cell death to the migration of cells, synaptogenesis, and the fine tuning of cellular and histological interactions.

443. In order to obtain much needed information of potential value under normal and accidental conditions of exposure to internal radiation sources, there is an urgent need to enlarge the data base on the metabolism and effects in utero of radionuclides, especially of those having practical importance, over a wide range of chemical forms and activity concentrations. Such studies should be carried out in relation to tissue dose, rather than intake concentrations, in order to facilitate generalized conclusions on dose-response relationships. More generally, better estimates of the actual doses absorbed by the fetus at various representative pre-natal ages could be very useful.

444. Finally, there is a need for inter-species comparisons of the radiation response of developing germ cells in various animals and both sexes for acute and long-term exposure.

Table 1

Approximate time of the beginning and end of the major developmental periods in some mammalian species
(days p.c.)
[U2]

Species	Pre-implantation	Major organogenesis	Fetal period
Hamster	0-5	6-12	13-16.5
Mouse	0-5	6-13	14-19.5
Rat	0-7	8-15	16-21.5
Rabbit	0-5	6-15	16-31.5
Guinea-pig	0-8	9-25	26-63
Dog	0-17	18-30	31-63
Man	0-8	9-60	60-270

Table 2

Correlations between menstrual age and crown-rump length according to [S55] or [J9]

Menstrual age		Crown-rump length according to [S55]	Crown-rump length according to [J9] a/
Weeks	Days	(mm)	(mm)
9	63	31	24
10	70	40	33
11	77	50	43
12	84	61	53.5
13	91	74	66
14	98	87	82.5
15	105	101	99.5
16	112	116	117
17	119	130	135
18	126	142	151

a/ This study was based exclusively on fetuses obtained from artificially induced abortions.

Table 3

Regression coefficients for fetal organ weights [B14]

		Coefficients a/			Standard error of estimate
		a	b	c	
Organ	N	(10^{-2})	(10^{-4})	(10^{-6})	
Brain	71	14.828	-0.126	-	6.127
Liver	75	6.097	-0.421	0.032	4.919
Lung	76	3.777	-0.286	0.016	3.475
Kidney	78	0.857	-0.016	0.003	0.842
Heart	76	0.592	0.029	-0.002	0.498
Thymus	76	0.073	0.042	-0.002	0.417
Thyroid	70	0.035	0.074	-	0.048
Pancreas	71	0.085	-0.002	-	0.118
Adrenal	76	0.478	-0.033	0.002	0.384
Spleen	75	-0.001	0.046	-0.003	0.244

a/ Coefficients for the polynomial regression formula $y = ax + bx^2 + cx^3$ where x is body weight and y is organ weight.

Table 4

Developmental stages in human embryos
[08]

Carnegie stage	Pairs of somites	Length (mm)	Age a/ (days)	Age b/ (days)	Features
1				1	Fertilization
2			1.5-3	2- 3	From 2 to about 16 cells
3			4	4- 5	Free blastocyst
4			5-6	5- 6	Attaching blastocyst
5		0.1-0.2	7-12	7-12	Implanted although previllous
5(a)		0.1	7-8		Solid trophoblast
5(b)		0.1	9		Trophoblastic lacunae
5(c)		0.15-0.2	11-12		Lacunar vascular circle
6		0.2	13	13-15	Chorionic villi; primitive streak may appear
6(a)					Chorionic villi
6(b)					Primitive streak
7		0.4	16	15-17	Notochordal process
8		1.0-1.5	18	17-19	Primitive pit; notochordal and neurenteric canals
9	1- 3	1.5-2.5	20	19-21	Somites first appear
10	4-12	2 -3.5	22	22-23	Neural folds begin to fuse; two pharyngeal bars; optic sulcus
11	13-20	2.5-4.5	24	23-26	Rostral neuropore closes; optic vesicle
12	21-29	3-5	26	26-30	Caudal neuropore closes; three pharyngeal bars; upper limb buds appearing
13	30- ?	4-6	28	28-32	Four limb buds; lens disc; otic vesicle
14		5-7	32	31-35	Lens pit and optic cup; endolymphatic appendage distinct
15		7-9	33	35-38	Lens vesicle; nasal pit; antitragus beginning; hand plate; trunk relatively wider; cerebral vesicles distinct
16		8-11	37	37-42	Nasal pit faces ventrally; retinal pigment visible in intact embryo; auricular hillocks beginning; foot plate
17		11-14	41	42-44	Head relatively larger; trunk straighter; nasofrontal groove distinct; auricular hillocks distinct; finger rays
18		13-17	44	44-48	Body more cuboidal;elbow region and toe rays appearing; eyelids beginning; tip of nose distinct; nipples appear; ossification may begin
19		16-18	47.5	48-51	Trunk elongating and straightening
20		18-22	50.5	51-53	Upper limbs longer at bent elbows
21		22-24	52	53-54	Fingers longer; hands approach each other, feet likewise
22		23-28	54	54-56	Eyelids and external ear more developed
23		27-31	56.5	56-60	Head more rounded; limbs longer and more developed

a/ According to [012] for stages 11-23; miscellaneous sources for stages 1-10.
b/ According to [J14].

Table 5

Birth prevalences of all isolated common congenital abnormalities per 1000 total births, based on a WHO study
[S38]

ICD code [W28]	Congenital anomaly	Average	Minimum	Source of data a/	Maximum	Source of data a/	Comments
740	Anencephalus and similar anomalies	1.1	0.1	LL	4.5	BE	Obvious territorial cluster in the British Isles
741.0	Spina bifida	0.8	0.1	M	4.2	BE	
742.0	Encephalocele	0.1	0.0	X	0.7	AL	
	Neural tube defects	2.0	0.6	C	9.0	BE	
745.0-747.9*	Congenital cardio-vascular malformations	6.1	3.2	BI	18.5	G	Very heterogeneous category. Various types should be evaluated separately
745.3-4*	Ventricular septal defect	1.7	0.4	BI	3.3	LE	
749.1-2	Cleft lip and/or cleft palate	1.0	0.0	ME CT	1.6	J	Ethnic differences: African: ~ 0.4 European: ~ 1.0 Oriental: ~ 1.9
750.5*	Congenital hypertrophic pyloric stenosis	2.0	0.1	SI	3.3	A	Ethnic differences: African: ~ 0.5 European: ~ 2.0 Oriental: ~ 0.4
752.5	Undescended testicle	1.2	0.0	X	12.2	BO	Diagnosis depends on time of birth and post-natal age
752.6*	Hypospadias	2.8	0.4	S	4.3	R	There are common minor variants
754.3	Congenital dislocation of hip	0.3	0.0	X	3.2	BO	Increasing trend owing to neo-natal screening
754.5-754.6	Varus, valgus and other deformities	2.7	0.4	C	20.9	PA	Very heterogeneous category. Various types should be evaluated separately
754.53*	Structural talipes different types equinovarus	1.3	1.0	S	3.0	L	
755.0	Polydactyly	1.1	0.0	ME	6.2	P	Obvious ethnic cluster
758.0	Down's syndrome	0.8	0.0	AL, CT	3.9	LL	Decreasing trend
550.1*	Congenital inguinal hernia	18.1	10.2	H	130.0	E	Not included in chapter XIV of the ICD

a/

A	Aberdeen, United Kingdom	LE	Leiden, The Netherlands
AL	Alexandria, Egypt	M	Manila, Philippines
BE	Belfast, United Kingdom	MA	Madrid, Spain
BI	Birmingham, United Kingdom	MC	Mexico City, Mexico
BO	Bogota, Colombia	ME	Melbourne, Australia
C	Calcutta, India	N	Newcastle, United Kingdom
CT	Cape Town, South Africa	P	Pretoria, South Africa
E	Edinburgh, United Kingdom	PA	Panama
G	Greenland	R	Rochester, United States of America
H	Hong Kong	S	Sweden
J	Johannesburg, South Africa	SI	Shiraz, Islamic Republic of Iran
K	Kuala Lumpur, Malaysia	SP	Sao Paolo, Brazil
L	London, United Kingdom		
LL	Llubljana, Yugoslavia	X	Several centres

Table 6

Birth prevalences of all isolated common congenital abnormalities per 1000 total births based on a Hungarian study [C18]

ICD Code [W28]	Congenital anomaly	Average	Etiology a/	Comments
740	Anencephalus and similar anomalies	1.1 (0.8)	MTM)	A decreasing trend in recent years (shown in parentheses)
742.0	Encephalocele	0.2 (0.2)	MTM)	
	Neural tube defects	2.9 (1.9)	MTM)	
745.0-747.9	Congenital cardiovascular malformations	10.6	H	Result of population screening
745.3-4	Ventricular septal defect	2.1	MTM	In a proportion of cases, spontaneous closure occurs
749.1-2	Cleft lip and/or cleft palate	1.0	MTM	-
750.5	Congenital hypertrophic pyloric stenosis	1.5	MTM	Only operated cases
752.5	Undescended testicle	3.6	MTM	After third month. This figure is ~ 35 in males at birth
752.6	Hypospadias	2.2	MTM	Trend is increasing
754.3	Congenital dislocation of hip	28.0	MTM	Only treated cases, i.e., without true abnormality
754.5-754.6	Varus, valgus and other deformities of feet	3.0	M	Mainly postural deformities
754.53	Structural talipes equinovarus	1.3	MTM	A true abnormality
755.0	Polydactyly	0.3	G	Not a common abnormality in Hungary
758.0	Down's syndrome	1.2	C	Verified by chromosomal analysis
550.1	Congenital inguinal hernia	11.4	MTM	Only operated cases

a/ C = chromosomal; G = genic, at least some part; H = heterogeneous; M = maternal; MTM = multifactorial threshold model; i.e., polygenic disposition interacting with a variety of environmental factors.

Table 7

Birth prevalences of some isolated moderately frequent congenital abnormalities per 1000 total births

ICD Code [W28]	Congenital anomaly	WHO Study [S38]					Hungarian study	Etiology
		Average	Minimum	Source of data a/	Maximum	Source of data a/	[C18]	b/
742.3	Congenital hydrocephalus	0.6	0.2	MC	2.0	AL	0.8	H
749.0	Cleft palate	0.2	0.0	X	0.5	ME	0.4	MTM (?)
750.3	Oesophageal atresia and stenosis and/or tracheo-oesophageal fistula	0.1	0.0	X	0.5	SP	0.2	U
751.2	Atresia and stenosis of large intestine, rectum and anal canal	0.2	0.2	ME CT	0.6	K	0.2	U
753.1	Cystic kidney	0.03	-		-		0.1	G_1
755.1	Syndactyly	0.2	0.0	X	0.7	J	0.3	G_2
755.3-5	Reduction deficiencies of limb	0.2	0.0	H P	1.8	SP	0.4	H
756.6	Anomalies of diaphragm	0.1	-		-		0.2	MTM
756.7	Exomphalos	0.1	0.0	X	0.5	MA	0.2	MTM

a/ Abbreviations explained in the footnote a/ to Table 5.
b/ G_1 = genic, at least type I and II; G_2 = genic, at least some part; H = heterogeneous; MTM = multifactorial threshold model.

Table 8

Percentage distribution of congenital anomaly groups
(modified from [V8])

Group of congenital anomalies	Authors a/							
	A	B	C	D	E	F	G	H
	Total birth prevalence							
	1.4	1.5	1.3	2.8	3.8	2.2	8.5	7.4
Central nervous system	26.3	13.3	21.0	11.6	5.7	7.7	8.5	4.2
Cardiovascular system	7.2	7.0	5.9	8.7	10.9	19.2	10.2	11.0
Cleft lip and palate	11.6	10.8	9.5	8.8	6.3	7.9	3.2	2.0
Alimentary system	5.4	4.9	4.1	8.7	7.4	7.9	7.3	3.8
Genito-urinary system	18.2	7.2	2.0	7.8	15.7	12.4	13.6	12.7
Limbs	27.2	53.0	38.0	35.9	36.8	28.1	39.2	41.2
Down's syndrome	3.5	3.6	6.5	-	5.7	6.3 b/	1.4	1.6

a/ A = Büchi, 1950 (reference given in [V8];
B = Stevenson et al., 1958 (reference given in [V8];
C = Stevenson, 1966 (reference given in [V8];
D = Klemetti, 1966 (reference given in [V8];
E = Villumsen, 1970 [W8];
F = Trimble and Doughty, 1974 [T10];
G = Myriantropoulos and Chung, 1974 [M45];
H = Czeizel and Sankaranarayanan, 1984 [C19].
b/ All chromosomal aberrations.

Table 9

Incidence of severe mental retardation in individuals exposed pre-natally to the atomic bombing of Hiroshima and Nagasaki a/

Data for the two cities have been combined and the cases distributed by gestational age at exposure and fetal absorbed dose, based on the T65 revised dosimetry b/

(Source of data: [04])
[This table is taken from III]

Ages	Dose categories (Gy) c/				
	> 0.01	0.01-0.09	0.10-0.49	0.50-0.99	1.00+
	Cities combined				
All gestational ages					
Subjects	1085	292	169	34	19
Retarded	9	4	4	6	7
Percent	0.8	1.4	2.4	17.6	36.8
0-7 weeks					
Subjects	210	55	26	2	2
Retarded	1	0	0	0	0
Percent	0.5	0.0	0.0	0.0	0.0
8-15 weeks					
Subjects	257	69	50	13	9
Retarded	2	3	4	4	6
Percent	0.8	4.3	8.0	30.8	66.7
16-25 weeks					
Subjects	312	86	45	15	5
Retarded	2	1	0	2	1
Percent	0.6	1.2	0.0	13.3	20.0
26+ weeks					
Subjects	306	82	48	4	3
Retarded	4	0	0	0	0
Percent	1.3	0.0	0.0	0.0	0.0

a/ Note that the frequency distribution by gestational age in this Table is slightly inconsistent with that given in [03] due to differences in grouping; these differences have little effect on the risk estimates. The data for the two cities are separately presented elsewhere [03, 04].

b/ The tissue gamma dose is taken to be equal to 0.42 times the gamma kerma plus 0.077 times the neutron kerma, the tissue neutron dose to be equal to 0.14 times the neutron kerma, and the total tissue dose to be the sum of the two individual tissue doses.

c/ The mean doses within these dose categories over all gestational ages are 0, 0.04, 0.23, 0.72, and 1.61 Gy, respectively.

Table 10

The relationship of mental retardation to absorbed fetal dose

(Source of data: [04])
[This table is taken from I11]

Gestational age	Cities combined					
	a	s_a	b (Gy^{-1})	s_b (Gy^{-1})	Deviance	P
All gestational ages	0.768	0.253	0.174 a/	0.041	3.78	0.29
8-15 weeks	0.917	0.567	0.404 a/	0.078	1.11	0.77
16-25 weeks	0.601	0.415	0.101	0.059	3.25	0.36
Relationship of mental retardation to dose: 'controls' excluded						
8-15 weeks	2.189	2.242	0.378 a/	0.089	0.61	0.74
16-25 weeks	0.409	1.055	0.106	0.068	3.20	0.20
Relationship of mental retardation to dose: 'controls' combined						
Pooled control	0.863	0.278	0.406 a/	0.077	1.11	0.77

The deviance, which is a measure of the goodness-of-fit of the model to the data, has three or two degrees of freedom depending upon whether the zero dose group is or is not included in the modeling; the P value is the probability (two tailed) of exceeding the deviance by chance under the null hypothesis. a is the estimated number (intercept) of cases of mental retardation (per 100 individuals) in the zero dose group and s_a its standard error; bD is the increase in the frequency of mental retardation, with dose D, expressed in grays (100 rad); and s_b is its standard error. Note that with the binomial distribution the deviance takes the form $2\{\text{Sum } z \ln(z/\hat{z}) + \text{Sum } (n-z) \ln [(n-z)/(n-\hat{z})]\}$ where the z's are the observed values, $\hat{z}$ the estimates of the p's under the complete model, that is when the p's are all different and match the data completely, and the $\hat{z}$'s are the estimates of the p's under the fitted model.

a/ Significant at the 0.001 level.

Table 11

Approximate length of the oogenetic period in the female a/ and approximate life span of gonocytes in the male b/ for selected mammalian species
(Data from [E2])

Species	Sex	Days	References as quoted in the original publication [E2]
Mouse	male	14	Erickson [E2] (1978)
	female	5	Peters (1970)
Rat	male	17	Huckins and Clermont (1968)
	female	6	Beaumong and Mandl (1962)
Guinea pig	male	50	Erickson [E2] (1978)
	female	25	Ioannon (1964)
Pig	male	158	Erickson (1964)
	female	77	Black and Erickson (1968)
Bovine	male	340	Erickson and Martin (1972)
	female	100	Erickson (1966)
Monkey	male	~1000	Van Wanegen and Simpson (1954)
	female	100	Baker (1966)
Man	male	~3500	Charney et al. (1952)
	female	120	Baker (1963)

a/ Time in days from the formation of the germinal ridge to the end of significant mitotic activity of the oogonia.
b/ Time in days from the formation of the germinal ridge to the appearance of the first differentiated spermatogonia.

Table 12

Relative leukaemia risk in retrospective studies of children dying of leukaemia after diagnostic irradiation in utero
[U3]

Age of leukaemics (years)	Years of deaths for leukaemics	Percentage of mothers receiving abdominal irradiation during pregnancy		Relative risk (95% limits in parentheses)	Ref.
		Leukaemics	Controls		
< 10	1953-1955	96/780 (12.3%)	117/1.638 (7.1%)	1.8 (2.4-1.4)	[S23]
< 10	1951-1955	20/70 (28.6%)	48/247 (19.4%)	1.7 (2.9-0.8)	[F6]
?	1955-1956	37/150 (24.7%)	24/150 (16.0%)	1.7 (3.7-1.0)	[K21]
?	1955-1956	34/125 (27.2%)	27/125 (21.6%)	1.4 (2.5-0.7)	[K21]
?	1950-1957	72/251 (28.7%)	58/251 (23.1%)	1.3 (2.0-0.9)	[K21]
?	1946-1956	5/55 (9.1%)	8/55 (14.5%)	0.6 (2.0-0.2)	[K28]
< 20	1940-1957	3/65 (4.6%)	3/65 (4.6%)	1.0 (12.0-0.6)	[M39]
< 20	1940-1957	3/65 (4.6%)	7/93 (7.5%)	0.6 (2.4-0.1)	[M39]
< 20	1940-1957	3/65 (4.6%)	2/82 (2.4%)	1.9 (40.0-1.1)	[M39]

Table 13

Crude annual incidence rate of leukaemia among children exposed in utero and controls, 1945-1979
[I3]

		Fetus total dose (Gy)				Total
		Not in city	0	0.01-0.4	> 0.5	
Subjects		2306	625	620	85	3636
Person-years		72444	19403	19078	2255	113180
Leukaemia cases		1	1	1	0	0
Rate (10^{-5})		1.38	5.15	5.24	0.00	2.65
90% confidence limit	Upper	6.55	24.46	24.87	-	6.85
	Lower	0.07	0.27	0.27	-	0.72

Table 14

Relative risk of leukaemia in pre-natally exposed children: summary of most recent studies a/

Study source	Years of incidence	Relative risk	Reference
United States	1947-1960	1.52 b/	[M53]
United States, white	1947-1967	2.88	[D15]
black	1947-1967	0.00	[D15]
United Kingdom	1943-1965	1.48 b/	[S24]
Finland	1959-1968	1.90	[S56]
Hiroshima/Nagasaki	1945-79	2.15 c/	[K4, I13]
United Kingdom, twins	1943-1965	2.20 b/	[M23]
United States, twins	1930-1969	1.60 d/	[H23]
United Kingdom	1980-83	1.33	[H30]

a/ Most cases and excess risks reported here occurred before the age of 10.
b/ Significant at 0.5% level or better.
c/ Relative risk from Japan was calculated from 1 case of leukaemia in 21,333 person-years at risk in irradiated groups compared with 2 cases in 91,847 person-years in control groups (Table 13) [I3]; the difference is not statistically significant.
d/ In this series, the relative risk of solid tumours was a non-significant 3.2; other series have found values less than the relative risk for leukaemia.

Table 15

Estimation of the acute LD/50 dose, minimal malforming doses, cell depleting dose, and doses for the human embryo based on compilation of mouse, rat, and human data [B27]

Age	Approximate minimal lethal dose (Gy)	Approximate LD/50 (Gy)	Minimum dose for recuperable growth retardation in adult (Gy) a/	Minimum dose for recognizable gross malformation (Gy)	Minimum dose for induction of genetic, carcinogenic and minimal cell depletion phenomena
Day 1	0.10	0.7-1.0	No effect	No effect	Unknown
Day 14	0.25	1.4	0.25	-	Unknown
Day 18	0.50	1.5	0.25-0.50	0.25	Unknown
Day 28	> 0.50	2.2	0.50	0.25	Unknown
Day 50	> 1.00	2.6	0.50	0.50	Unknown
Late fetus to term		3.0-4.0	0.50	> 0.50	Unknown

a/ Estimates for maximum dose effective in reducing body weight. Specific organs or measurements may be more or less sensitive.

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