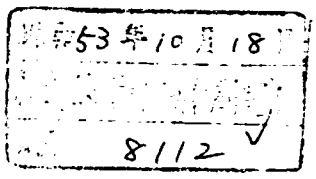
1977年報告

SOURCES AND EFFECTS OF IONIZING RADIATION

United Nations Scientific Committee
on the Effects of Atomic Radiation

1977 report to the General Assembly, with annexes



UNITED NATIONS
New York, 1977

NOTE

The report of the Committee without its annexes appears as Official Records of the General Assembly, Thirty-second Session, Supplement No. 40 (A/32/40).

In the text of each annex, Arabic numbers in parentheses are references listed at the end.

UNITED NATIONS PUBLICATION
Sales No. E.77.IX.1
Price: $U.S. 28.00
(or equivalent in other currencies)

Produced by UNIDO, Vienna

ANNEX J

Developmental effects of irradiation *in utero*

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Introduction

1. Since its 1962 report (344), the Committee has not comprehensively reviewed the data on pathological and teratological effects induced by irradiation of the mammalian embryo and foetus, with the exception of the effects on the developing central nervous system, which were considered separately in the 1969 report of the Committee (345). Much information has accumulated in the meantime; obtained mainly in animal experiments, it could be of help, if not for the prediction, at least for the interpretation, of the human experience. Additionally, reassessments of earlier observations and some new data in man have become available. This annex therefore reviews the effects observed in various mammalian species following exposure *in utero*, with the aim of extracting information relevant to the assessment of radiation effects in man. It deals exclusively with somatic effects.

2. Radiation exposure of the mammalian conceptus *in utero* gives rise to a large variety of short- and long-term deleterious effects. For many of them, especially those following irradiation during late pregnancy, the pathogenesis is similar to that of lesions seen after irradiation of animals in the extra-uterine life. There are, however, effects which may be viewed as the typical sequelae of irradiation of a developing organism, which is characterized by major processes of differentiation leading to the structural and functional organization of organs and tissues. The complex morphological and physiological events leading to the development of a mature animal may be disturbed or disrupted by radiation, and the consequence is the production of malformations of different nature and degree. Some of these defects are so profound as to be incompatible with the animal's life *in utero*; some can easily be recognized morphologically at birth; others cannot even be scored at the microscopic level and are only manifested at a later stage as functional deficiencies.

A. DEFINITION OF MALFORMATIONS

3. There is some lack of uniformity in the definition of congenital malformations owing to the large variety of these lesions. Brent (27) regards as malformations all permanent defects (morphological, histological or biochemical) which an organism cannot repair during the course of its normal growth and development. Warkany (356), on the other hand, draws a distinction between congenital anomalies and congenital malformations: he regards the first type as a broad category covering all microscopic, biochemical and functional disturbances seen at birth, and reserves the second term only for the gross structural effects in the new-born animal. According to Stevenson (334), the characteristic features of congenital malformations are their production during intra-uterine life (even though they may be recognized only at a later stage) and their macroscopic anatomical nature.

4. It is not surprising that this absence of uniformity in the definition is reflected in different estimates of induction rates as a function of time or radiation dose. It is largely a matter of definition whether growth retardation of an otherwise normal animal should or should not be regarded as a malformation, or whether malformations should comprise changes in the life-span of animal populations, or whether one should include the malformations seen in animals dying *in utero* or in stillborn animals and which therefore are never scored in live animals.

5. There is no doubt that when these problems are approached experimentally the area of concern should be carefully defined, so that the design, execution and interpretation of the work may be carried out properly. However, when reviewing a large body of experimental data, the acceptance *a priori* of firm definitions might lead to the exclusion of important information. Since it is the Committee's intention to consider all evidence at hand which might be of help in interpreting the scanty information available for man, it follows that no information should be disregarded. Data on genetic damage are covered in another section of the Committee's report (Annex H) and data on behavioural effects of irradiation *in utero* have also been reviewed previously (345).

B. PREVIOUS REVIEWS

6. Taking such a wide perspective does not imply that the present contribution attempts to review extensively most of the work in radiation teratogenesis.

which would obviously be an impossible task. It does not consider, for example, much of the early work on animals and man, which made important qualitative contributions but was often limited to very few observations or lacked quantitative definitions of the relevant physical and biological parameters. Nor does it include the work on animals that are phylogenetically far from the mammals, such as birds, amphibia, fishes and marine organisms. The subject of embryonic irradiation has been discussed with great care and scientific authority in many published reviews (52, 260, 262, 264, 362, 295, 230, 27, 109, 39, 127, 121, 370, 136, 170, 303, 92). An updated bibliographic compilation of most published contributions was also made available to the Committee (336), as well as two reports on the teratogenic action of external (178) and internal (178a) radiation sources, summarizing relevant work in the Union of Soviet Socialist Republics. Previous reports of the Committee have also considered general (344) or specialized aspects of radiation teratogenesis (345). All these publications may be consulted for background information.

C. PRESENT REVIEW

7. The developing embryo has some inherent biological characteristics that have always appealed to the radiation biologist (295). Differentiation of major organs and tissues takes place in an orderly sequence at well defined times after copulation or conception, the time of which can be established to a fairly good approximation (see paragraphs 26 and 27). Waves of cell division, differentiation and migration occur during organogenesis at certain times and, since radiation is particularly effective at these times, it is possible to induce selectively malformations in organs or anatomical regions. In addition, the dependence of the mammalian embryo on the mother allows the analysis of abnormalities up to very advanced stages of development that would not be compatible with life in other lower animals.

8. Although radiation-induced malformations are non-specific, in the sense that they can be produced by other teratogenic agents, the use of radiation in experiments offers some advantages over, for example, chemical agents. In fact, radiation from external sources can be administered to the embryo in precisely measurable quantities within very short times, whereas chemical substances must cross the placental barrier, and their kinetics of uptake and clearance result in uneven and less controlled exposures to the effective drug level. Furthermore, there is no selective concentration phenomena since radiation is distributed uniformly over the irradiated embryo; the selectivity of the teratogenic response can therefore be related to the sensitivity of the different structures at the time of irradiation. As with chemical substances, radiation may not be administered to the embryo without concomitant exposure of the mother or, at least, of the pregnant uterus. However, the indirect effects on the embryo, in the case of radiation, are relatively minor (see paragraphs 265-270).

9. For all these reasons the irradiated mammalian conceptus has also been a favourite biological system for the experimental embryologist. It is quite clear that most of the work reviewed has actually been carried out with the ultimate purpose of studying the mechanisms of development, rather than of analyzing the effect of the main radiobiological variables. There is insufficient quantitative information on dose-effect relationships and on the influence of dose rate and radiation quality, not only for the irradiated human embryo, as might be expected, but for experimental mammals as well. Such information lies often buried in large collections of systematic data on kinetics and morphology of malformed tissues.

10. Contrary to most previous reviews, this one will therefore attempt to extract the relevant radiobiological information from the published experimental work, giving particular emphasis to the data of prospective use in the assessment of radiation effects in the human embryo.

I. METHODOLOGY AND TECHNIQUES

A. GENERAL DISCUSSION

11. Before reviewing experimental data in detail it seems appropriate to examine some general questions of methodology and technique relevant to the study of the teratogenic effects of irradiation. Consideration of these questions is essential for the following purposes: *(a)* to illustrate some of the variables or conditions known to influence the induction of malformations and their scoring in laboratory animals; *(b)* to point out the confidence limits and the degree of precision of the data obtainable; *(c)* to warn against generalizations which may not apply to all biological materials and experimental conditions; and *(d)* to set in an appropriate perspective the scanty knowledge available in man.

12. In regard to the animals in which experimentation has been performed, the mouse appears by far the most thoroughly studied, followed by the rat, the rabbit, the hamster and the dog. Data on other species are only occasional. The data in humans represent a separate category and will be treated as such, not only because of their importance but also because little control of the experimental variables can be exerted in this case. Among the biological variables, the embryonic and foetal ages at irradiation have been investigated very extensively in many species. The applicability of these data to man, through a comparative evaluation of the times at which certain important stages of development occur, is also an important issue for discussion. The physical variables affecting the teratogenic effect (dose, dose rate, fractionation, protraction, type and energy of the radiation used) have only been examined in a few special cases. The conditions of exposure which apply to the human case and to animal experimentation should therefore be discussed, in order to ascertain if reasonably uniform sets of data are being compared.

B. BIOLOGICAL FACTORS

1. Genetic homogeneity

13. Certain characteristics are required for the selection of the animals used in embryological experiments. According to Jacobson (136) these are

genetic homogeneity, high fertility, large litter size, and a high degree of sensitivity to the teratogenic agent. These requirements may in some cases be in contrast to each other, so that the optimum experimental condition in any given case is often the best compromise between the various needs.

14. The high fertility and large litter size are conditions obviously favouring the rapid collection of statistically large experimental samples; they are inherent characteristics of the animal species and strains that are desirable choices for experimentation. Concerning genetic homogeneity, even in the absence of knowledge on the relative importance of constitutional and environmental factors in the induction of malformations, there is certainly an advantage in selecting the genetic background as uniform as possible. It follows therefore that the animals should be as homogeneous as possible in their genetic constitution, although some inbred lines may show a high natural incidence of specific malformations. The susceptibility to radiation-induced teratogenic effects is very high in inbred strains, but this advantage is often balanced by decreased reproductive and somatic vigour. In such cases hybrid animals obtained by crossing inbred strains may be the solution of choice to combine vigour and genetic homogeneity. Several approaches to this problem, all equally justifiable, appear to have been followed in the work reviewed.

2. Maternal factors

15. Quite aside from the response of the embryo to any teratogenic agent, there are certain biological characteristics of the animals used that must be taken into account when planning experiments. The quantitative features of reproductive performance and their variability are the most obvious ones, and some data are available on these points. Analyzing the interstrain differences in the breeding capacity of six inbred strains, Ehling (80) has drawn attention to the fact that the intraspecies and interspecies variabilities are of comparable degree. In the mouse, for example, the mean number of offspring per fertile female ranged from 20.5 to 73.4 in various genotypes examined; the embryonic survival varied between 57.1 and 75.6 per cent and the mean number of litters per female between 5.9 and 11.2. Differences in reproductive performance between F_1 hybrid females from reciprocal crosses appearing after irradiation at 50 R[1] have been attributed to a genetically controlled maternal factor (81).

16. But variability in reproductive performance is very high even within the same strain. According to Rugh and Wohlfromm (291), the first litter is always the smallest and the litter size tends to increase in the following three litters. The average litter size is greater among multiparous than among primiparous females of comparable ages and the incidence of abnormal offspring, although never very high in control mothers, tends to increase in successive litters. Many authors therefore tend to standardize experimental conditions by using second-litter animals for analysis of teratogenic responses, since the first litter usually has high mortality and small size. Correlations have been reported (136) between litter size at first delivery and litter size, conceptus number and weight of offspring of the second pregnancy. These correlations could to some extent guide the experimenter in the selection of suitable experimental animals in second litters on the basis of the outcome of the first.

17. Regarding the incidence of malformations after irradiation, the previous reproductive history of the mother appears to be of some importance. Thus, the incidence of intra-uterine deaths and resorptions after 200 rad received at 8.5 days post-conception (p.c.) is almost double among breeders 14 months old than among primiparous young females (287). Similar observations have also been made after doses of 2-32 rad (175). The incidence of gross aberrations of the central nervous system, which is 30 per cent higher in old females, is reduced after correction for *in utero* mortality, but a slight excess of malformations of the order of 3 per cent is still evident in offspring from old mothers (287). In another report (290) primiparous females, even though of the same age as the multiparous, were shown to produce more abnormal embryos. These and other data emphasize the need for careful control of the mother's age and of her past breeding experience in order to ensure good experimental material and uniformity in the teratogenic response. It may be added, however, that in a careful and extensive study by Jacobsen (136) of all these maternal factors, they were assigned a relatively minor influence, in any case not sufficient to result in gross misinterpretations of the dose-effect relationships for the induction of malformations. It may also be mentioned that the radiation history of the female prior to mating may alter some of the reproductive parameters, sometimes with an increase of the litter size (104). Other factors, e.g., immunological incompatibility between the mother and foetus, are known to alter the reproductive performance of mammalian species, but their effect, particularly in regard to the induction of malformations, has not been made the object of special consideration in this Annex.

3. Homozygous and heterozygous animals

18. The influence of genetic constitution in the production of malformations and the relative susceptibility of homozygous *versus* heterozygous animals have received some attention, particularly in mice, where the availability of inbred strains make such analyses easier. Russell and Russell (303) tested the relative sensitivity of three different but genetically uniform mouse populations ((C57 X NB)F_1, BALB/C and 129), taking as the end-point the incidence of animals having 13, more than 13 or less than 13 ribs on each side of the body. They concluded, on the basis of the variation between and within strains of the control animals, that the expression of this character results from a continuous array of variation of the underlying process. They therefore emphasized the need for great caution in drawing conclusions about relative sensitivity with regard to characters having a continuous variability. In fact, particularly under such conditions, a change in the

[1] Throughout this Annex, the radiation units quoted are the roentgen (R) or the rad, as they appear in the papers reviewed. No attempt has been made to express the frequency of radiation effects on a common basis of unit absorbed dose.

end-result would not necessarily indicate a corresponding difference in primary radiation damage (see paragraph 287).

19. Dagg (68) examined the teratogenic response of three strains (BALB/cGn, C57BL/ks and 129/Rr) and some of their hybrids, showing that the differences in malformation response between strains observed after irradiation at 11 days p.c. is not found when the irradiation occurs at 10 days p.c. or when the embryos are irradiated at approximately equal crown-rump length. Thus, the differences observed may be due to different developmental (as opposed to chronological) age of the embryos at the time of exposure rather than to an intrinsic difference in susceptibility.

20. Nash and Gowen (218) followed the post-natal growth response of three inbred strains (Ba, K, and S) and all their hybrids, including reciprocal crosses, at several gestational ages and after various doses. They found evidence that developmental differences are under strong genetic control. These differences may to some extent be related to even minor strain-related changes of the developmental age at the time of irradiation, but they can be exerted in part through the ability of single cells and organs to repair radiation damage. A similar influence of the genetic background was also found with respect to the duration of life-span (219); here again the genotype was shown to condition embryological development at irradiation, and the differences between strains were particularly evident at high irradiation doses given at late pregnancy stages.

21. In a review centred on the question of outbred *versus* inbred animal strains in experimental teratology, Smithberg (327) has considered all relevant problems exhaustively. He concluded that the selection of the most appropriate experimental animals cannot be made on the basis of general principles, but rather depends on the specific aims to which research is directed; in different instances and for different purposes, either genetically pure or outbred animals may be chosen with equally good justification.

22. The available reports almost unanimously show that the heterozygous condition confers a higher degree of resistance to the teratogenic action and to the killing effect of radiation *in utero*. To cite just some of the evidence, Rugh and Wohlfromm (288) have reported an increased radioresistance of hybrid embryos (C57BL/6 X CF1) exposed to 200 R at 8.5 days p.c., by comparison with the parental strains. At 18.5 days, in fact, only 5 per cent of the implants of the C57BL animals were normal, while for the CF1 and the hybrid mice the respective values were 38 and 44 per cent. The small difference between CF1 and hybrids was attributed to heterosis of the hybrids. Dagg (68) reported that hybrids from crosses of C57BL and BALB/C were more resistant to lethal effects and to deforming effects in the foot, palate and tail than homozygous C57BL embryos. Jacobsen (136) refers also to a higher degree of sensitivity of homozygous HC mice relative to (HC X C3H) hybrids. Finally, a differential response between inbred and hybrid mice of three genetic stocks (BALB, K and S) was shown by Nash (217) in regard to both induction of malformations and lethality (neo-natal and early post-natal). In all cases inbred genotypes responded to a greater degree than hybrids.

23. It may thus be concluded that the genetic constitution has an influence on the degree of the observed lethal and teratogenic responses. But just how much of the differential response might be due to intrinsic variation of sensitivity or repair in various species and strains and how much, on the other hand, might be attributed to small variations of the developmental age at irradiation remains unclear. Variations of the rate of development are also genetically controlled, but their effect is indirect and due to rapid changes in embryonic sensitivity in the course of differentiation. The heterozygous conditions seem invariably to result in a higher resistance to lethality and malformations, but the need to irradiate similar developmental stages for meaningful quantitative comparisons remains valid. Finally, the type of effect scored may affect the conclusions about relative sensitivities, especially when it is based on some continuous variable rather than on discrete morphological traits.

24. The position of the implants in the uterus is of relatively minor importance in relation to the occurrence of abnormalities, a question that has been examined by Hashima (114), Trasler (342) and Jacobsen (136). Hashima reported that offspring in the ovarian position of the uterine horn had a significantly reduced weight. Trasler found that embryos in the same position had an incidence of cleft palate which was higher than in all other positions. On the contrary, Jacobsen showed that embyros in the intermediate position were apparently at a disadvantage when the weight of offspring and the placental weight were taken as indicators of the optimal uterine environment. The incidence of skeletal malformations and resorptions were unrelated to the uterine position. These results, although contradictory in the various experimental conditions tested, show nevertheless that the position of the embryos in the uterus may perhaps affect the developmental potentialities and the malformation incidence of the irradiated embryos.

25. Important quantitative differences between experiments performed in summer and in winter were also reported by Jacobsen (136). In the experience of this author, the seasonal variability was so high that it would have masked the effect of doses up to 100 rad had the results not been evaluated separately for the two seasons. In general, summer samples were found to be less radiosensitive. It follows that experimental data must be tested against control groups run at the same time.

4. Timing of pregnancy

26. There is one aspect of breeding technique that requires special attention: the accurate timing of mating and fertilization. In rodents, successful mating is followed by the appearance of the vaginal plug; the time of scoring of this sign is often the starting point for calculations of the embryonic and foetal ages, especially in mice (266). However, many workers consider this time as day 0 and others as day 1 after fertilization, thus

creating inconsistencies among their experimental series. In rats, the presence of sperm in the vaginal canal is also used as an indication of mating (32). Useful and up-to-date information on the reproductive physiology of various experimental animals for an accurate timing of pregnancy may be found in the following references: mouse, 328, 266; rat, 223; rabbit, 109; dog, 6; monkey, 249.

27. Owing to differences in mating techniques used by various laboratories and because of the large time variability between ovulation and fertilization, there are usually differences in the estimation of the conception time and therefore of the gestational age. In some instances, gestational age is measured from the observed or presumed time of coitus and in other cases from the presumed time of fertilization or conception, calculated from the time of coitus or estimated back from the time of scoring the vaginal plug. However, the sacrifice of some experimental animals at irradiation time followed by direct examination of the conceptus, measurements of the length of embryos or observation of external morphological features, affords a better timing of the developmental stage at the time of irradiation (299). Such procedure is, however, carried out only rarely. Owing to the uncertainties referred to above, most estimates of embryonic age should not be taken to be more accurate than about ± 0.5 day, even under the best-controlled conditions. It should also be mentioned that marked differences in apparent age may be seen within embryos of the same litter, especially at the earlier ages (241, 4), and that there is therefore a limit to the improvement of timing control (135).

5. Periods of maximum sensitivity

28. The need for accurate control of the gestational age in experimental radiation teratogenesis stems from the evidence that there are certain well defined "critical periods" in embryonic development when some types of malformations can be produced more readily than others. The concept of critical period was first developed in 1911 from observations on fertilized amphibian eggs (14). Although repeatedly discussed in the following years (337), its importance was not fully and immediately realized. Actually, many of the earliest observations did not sufficiently specify the age at which the embryonic or foetal material had been exposed. The first systematic investigation of this concept in mammals was made by Job *et al.* (139) in 1935. They showed that exposing rats to 90 R at day 9 of gestation produced mainly hydrocephalus, whereas defects of the eye and the jaw were preferentially induced by irradiation on day 10 and 11, respectively. In this work the correlations between teratological effects produced at some specified times and the stage of differentiation of the relevant embryonic tissues at such times were clearly established. It was also pointed out that rather low doses are needed to obtain "specific" teratogenic responses and that "specificity" does not imply that some malformations can only be produced at certain times but rather that a given dose is more effective at some stages.

29. Later experience has shown that some malformations are more likely produced at certain times in pregnancy and that the period of sensitivity may be broadened by raising the dose. These observations have also indicated that susceptibility to malformation induction is not sharply defined in time but extends to a lesser degree before or after the critical period and that accurate mapping of these critical periods cannot be made at high doses (295). The data of Russell (295, 296), Rugh (261) and Rugh and Grupp (278) showed conclusively that these basic concepts hold generally true for many types of abnormalities in the mouse; Warkany and Schraffenberger (357), Wilson (362) and Brent (27) extended this notion to the rat. It is now commonly accepted that there are "stages of maximal sensitivity" in the teratogenic response to radiation (137). This new terminology recognizes that such periods cannot be defined for all doses of radiation and is in agreement with the definition of the "critical period" for teratogenic changes given by Russell (295) as the "developmental interval during which radiation must be applied to produce that change if the dose of radiation is the lowest one that gives a detectable incidence of that change".

C. COMPARATIVE MAMMALIAN EMBRYOLOGY

30. In view of the existence of periods of high sensitivity for the induction of certain malformations, which can be traced back to important stages in the organization of the relevant structures, it is important to study the comparative embryonic development of the different experimental animals. This comparative study is necessary to be able to generalize experimental findings and also to extrapolate results from one species to another. Such an exercise in comparative embryology has been attempted many times but the results are necessarily approximate and imprecise.

31. The general pattern of embryonic differentiation, which is qualitatively similar in all mammals, makes it possible to specify the approximate time of occurrence of certain easily recognizable ontogenic events, as shown in table 1. This table summarizes pre- and post-implantation events in six mammalian species used in radiation teratogenesis on the basis of numerous publications. It will be noted that the occurrence of certain events may be timed rather precisely, especially at the very early embryonic stages. However, as pregnancy proceeds and the structure of the embryo becomes increasingly complex, there is no single embryonic event for which a comparative analysis can be performed on the basis of objective parameters. The number of somites may be used as a possible reference of staging, but it remains true that the time of appearance of each structure in the embryo has a mean that is difficult to measure and a variance that probably increases with the age at which the structure originates (241).

32. Besides the inherent variability of the onset of the various structures, their development in various animal species may be advanced or retarded with respect to the development of the whole embryo. Therefore, the appearance of single structures cannot be accepted as a valid reference. It is instead necessary to make the assessment on the basis of several relative measurements referring to various systems (nervous, digestive, heart and circulatory), a procedure adopted, for example, by

TABLE 1. DEVELOPMENT OF EMBRYOS IN SOME MAMMALIAN SPECIES

Age (days p.c.)	*Mouse*	*Rat*	*Hamster*	*Rabbit*	*Dog*	*Man*
1	2 cells			2 cells		
1.5	4 cells		cleavage	4 cells		2 cells
2	5-8 cells			8 cells		4 cells
2.5	9-16 cells			16 cells		
3		4 cells		morula		5-8 cells
3.5	blastocyst	8 cells	early blastocyst	early blastocyst		9-16 cells
4		morula				
4.5	implantation		late blastocyst	late blastocyst		blastula
5		blastula	implantation			
5.5				implantation		
6	organogenesis	implantation				implantation
6.5	primitive streak		primitive streak			
7	head process		5 somites			proamniotic cavity
7.5			neural groove	neural groove 1-4 somites		
8	first somites	egg cylinder	organogenesis	organogenesis		
8.5			optic vescicle	heart formation	early blastocyst	
9	liver diverticulum	primitive streak	20 somites	neural tube closed		
9.5	anterior limb bud	neural plate		torsion of embryo		
10		first somites	limb bud formation			
10.5	posterior limb bud			anterior limb bud		
11		10 somites				
11.5			open eyelid			
12.5			closed eyelid	forelimb buds	late blastocyst	
13						germ layers, extra-embryonic membranes
14	foetal period					
15				foetal I period		
16		foetal period	birth	separation of digits		primitive streak
17					implantation	
18						head process first somites
19				palpebral fissure closed		
19.5	birth				primitive streak	
20				foetal II period		
20.5						appearance of heart
21					early somites	neural groove
21.5		birth				circulation of blood
22					limb buds	liver diverticulum
22.5						optic sulcus
23					15 somites	
25					tail bud	torsion of heart tube
28					end of major organogenesis	primary brain, G.I. tract and derivatives
30						optic vescicle
31.5				birth		
32						major organization of heart structures, limb buds
34						optic vescicle into optic cup, lens formation, lung buds
35					early foetal stage	
36						thickening of retinal layers
37						arm, forearm and hands recognizable
40						outline of digits (hand)
43						eyelids appear
60						definite limbs established
63					birth	↓

Sources: 3, 6, 22, 59, 92, 102, 105, 108, 195, 223, 225, 241, 245, 247, 262, 266, 295, 328.

Otis and Brent (241). Following such a rationale, these authors have constructed a table and a graph for the estimation of equivalent ages in mouse and human embryos, showing that the rates of development in the two species cannot be related by any simple continuous function. The estimates are certainly affected by substantial uncertainties but provide some average estimate of approximate age equivalence based on the actual developmental stages in various embryos. Rugh (266) has reported some data on the age equivalence of mouse and rat embryos. Using this information, it is possible to establish correlations of embryonic ages

between the species as shown in table 2. It should not be expected that such correlations would apply to minor details of the embryonic development; on the other hand, they certainly serve the useful purpose of allowing rough comparisons of pregnancy stage and embryonic age between these species. The approximate embryonic dimensions of the three species are also given in table 2 for reference purposes.

TABLE 2. EMBRYONIC AGE AND GROWTH DATA IN SOME MAMMALIAN SPECIES

Mouse		*Rat*		*Man*	
Age (days p.c.)	*Length (mm)*	*Age (days p.c.)*	*Length (mm)*	*Age (days p.c.)*	*Length (mm)*
0				1	
1		2		2	
2		3.25			
3		4		3	
4		5	0.13	4	
4.5		6	0.17		
5		6.75		5-6	
5.5		7.25	0.23	7-8	
6		7.5		9-10	
6.5		7.75		11-13	0.15
7		8.5	0.5-0.6	14-17	0.40
7.5		9	0.8	18-20	
8		10		20.5	1.5
8.5	2.0	10.5	2.0	22	
9	2.2			25.5	2.4
9.5	3.3	11	6.0	26	3.0
10	3.8	11.5		27	
10.5	5.2	12.125		28.5	4.2
11	6.2	12.5	12.8	31	4.5
11.5				33.5	7.0
12	7.2	13	14.0	36	
12.5	8.9	13.5		36.5	9.0
13	9.4	14.5	17.6		
13.5	9.8			38	12.0
14.5	11.2	15.5		47	17.0
14		16	19.0		
15.5	13.7			65	40.0
16		17-18	29-32		
16.5	16.1			84.5	
17-19	19.8	19-22			

Sources: Same as table 1.

D. IRRADIATION TECHNIQUES

33. The usual recommended procedures for the experimental irradiation of small and medium-size animals (130) are fully applicable to experiments with pregnant animals. In special instances, irradiation of the pregnant uterus or of a single uterine horn with shielding of the rest of the mother's body or parts of it has been adopted in mice (240) and rats (362, 32), particularly to elucidate the direct action of radiation on the conceptus, as opposed to possible indirect effects mediated by irradiation of the mother. Such procedure allows also a proper comparison between irradiated and control animals belonging to the same litter. Specific problems encountered in the irradiation of the foetus by internal emitters are discussed in chapter V.

E. LETHAL EFFECTS

34. Death of the offspring appears to be one of the most widely reported effects following irradiation *in utero*, and the work reviewed covers embryonic, foetal, and neo-natal mortality in several animal species.

35. Pre-natal mortality has been studied by means of several indicators, including the complete interruption of pregnancy, the percentage of resorbed conceptuses, the percentage of embryos alive pre- or post-partum, and the average litter size before or after birth. In a few instances tentative estimates of the LD_{50} at or just before term have been reported; these estimates can more readily be compared with data on post-natal whole-body irradiation. Neo-natal mortality and in some instances the lethality within the first month of delivery have also been reported. Finally, there are data from animals irradiated *in utero* and followed for the rest of their post-natal life for the purpose of estimating long-term parameters related to the time and mode of death.

36. The present report does not discuss the merits or disadvantages of the different ways of measuring lethality and their possible relationships; they are part of the experimental methodology under which the reviewed observations have been made. It should however be realized that even apparently minor variations of technique may yield quite different numerical estimates, even though they might reflect basically the same end-effect, namely radiation-induced lethality. For example, scoring dead offspring prior to or after birth may lead to quite different conclusions, since parturition trauma and mother cannibalism may modify the results profoundly. When reviewing the experimental data, the above comments should be kept in mind to evaluate correctly the reported death rates.

F. DISTURBANCES OF GROWTH

37. Although there may be doubts as to whether or not abnormal growth or growth retardation should be included among the radiation-induced congenital malformations, these effects have been widely described in animals and man following intra-uterine exposure. As in previous reviews of the Committee (344, 345), these effects are dealt with in the present report.

38. A review of the foetal, maternal and environmental factors that could affect foetal development has been published by Brent and Jensh (37). This publication deals mainly with growth retardations in the human foetus and discusses at some length the applicability to man of data obtained in polytocous experimental animals. It also reviews the relationship between teratogenesis and growth retardation, pointing out that although teratogenesis may be viewed as a differential growth disturbance of some tissues and both effects are often elicited by the same teratogenic agent, there are reasons to consider them separately at the present stage of knowledge. Whatever the etiology or the pathogenesis of growth retardation may be, it can be essentially defined as the failure of the foetus to maintain its normal expected growth potential at any gestational age, as measured by some parameter of foetal growth such as its weight or length. Frequently foetal growth retardation leads to growth defects in the extra-uterine life. This is particularly the case when retardation is induced by radiation.

G. MALFORMATIONS

1. Observation time

39. The time at which teratological effects should be examined is of great methodological relevance and is obviously dependent on the effect under study. There are effects that can only be observed under certain conditions and, in general, the magnitude of the effects is critically related to the observation time.

40. If lethality during the intra-uterine stages (pre- or post-implantation) is the end-point of interest, mortality must definitely be scored before birth. Mortality immediately after birth cannot be expected to reflect mortality *in utero* because the trauma of delivery and cannibalism may seriously affect the estimates. Alternatively, neo-natal, 30-day or long-term mortality may be the relevant end-points, in which case the observations should be conducted at the appropriate times. If growth retardation is the end-point, the selection of suitable conditions of scoring must take into account that the percentage weight reduction increases during the first few days after irradiation (362) and that the time for maximum effect is probably a dose-dependent variable (294, 362).

41. When studying the incidence of malformations, some authors score them after delivery (294, 280), but most workers prefer to sacrifice the mother just before term and to observe the foetuses *in situ*, in order to collect information that would otherwise be lost (position of the foetuses, presence of resorptions, placental form and weight, presence of intra-uterine bleeding etc.). Also, cannibalism practised preferentially on malformed offspring would alter quantitative estimates. The technique of sacrificing the mother before birth is adequate and accurate for the study of most types of malformations. However, there is no general rule for selecting the best observation time, which depends on the particular end-point under study.

2. Techniques of observation

42. Quite different data may result from the experiments, depending on the technique of examining the embryos. For this reason few papers among those reviewed are entirely comparable. Some investigators have examined, in more or less detail, only the external features of the foetus. Others have dealt particularly with special types of malformations (e.g., of the nervous or skeletal system) or have given elaborate morphological descriptions of their findings, sometimes with few numerical incidence data. Others again have been mainly interested in the pathogenesis of malformations and have therefore examined histologically the embryos soon after irradiation, often without relating the histological changes to the induction rate of malformations at later stages.

43. The combined effect of the large variety of experimental techniques, the differences between species and strains, the difference of gestational age at irradiation and the wide range of types of radiation and doses utilized, render any quantitative comparison of all the data virtually impossible. Reviews must therefore rely on the internal consistency of a single experimental series in order to prove certain points of interest. Under these conditions, only generalizations of gross qualitative observations are possible.

44. Detailed procedures for autopsy of irradiated foetuses have been published (294, 297). In general, they involve necropsy of the mother with careful examination of the uterus, foetal membranes and resorptions and examination and weighing of the placenta. The external examination of each foetus consists of a number of morphological characterizations and measurements: foetus shape and length; tail shape and length; morphology of anus and genitalia; abnormal posture of limbs; number, shape and position of digits; shape of head, jaw and mouth; exencephaly; position, size and morphology of ears, eyes, nostrils and nasal septa; presence of spina bifida, exomphalos and ventral hernia; etc. Each foetus is then eviscerated carefully for the macroscopic study of morphology and position of thoracic and abdominal viscera. If needed for examination of skeletal changes, the foetus may subsequently be processed by fixation, staining and clearance of the carcass. A large variety of morphological observations have been carried out on the embryo and foetus, from the simplest measurements of length and weight and conventional light microscopy to the most refined electron microscopical observations.

H. STATISTICAL EVALUATION

45. Statistical treatment of the data obtained in radiation teratology experiments does not usually require sophisticated techniques. Unless subtle questions of correlations between types and degrees of abnormalities are examined, simple statistical techniques for comparison of means and distributions are adequate. The papers reviewed only seldom refer to experiment design on the basis of knowledge of the experimental material or of the previous pilot work in relation to the experimental variables to be examined. In many cases the number of observations is small and, as a consequence, the statistical significance of the data is rather weak. This may be explained by the difficulties in obtaining appropriately timed and controlled embryonic material for irradiation, requiring a special organization of the animal quarters, special training of personnel and highly specialized techniques of observation. But it may also be attributed in part to the predominantly descriptive character of traditional embryological research. Experimental data often include extremely detailed recording of minute abnormalities, reported sometimes without appropriate homogeneous grouping and often compounding major malformations with trivial changes. For these reasons the Committee feels that information of a more gross nature, such as the fractional incidence of grossly malformed foetuses, might often be more informative for the purpose of assessments of the effects of radiation.

I. METHODOLOGY AND TECHNIQUES IN HUMAN INVESTIGATIONS

46. The wealth of information provided by well planned research on experimental animals far outweighs the information derived from observations in man. Many

of the human data are inadequate on account of one or more of the following reasons: *(a)* insufficient knowledge of the developmental stage at irradiation; *(b)* lack of precise estimates of the absorbed dose in the embryo; *(c)* malformations can be scored only after birth and on the basis of medical examinations and laboratory data; *(d)* the numerical limitations of the observed samples, often consisting of single observations, and *(e)* the difficulty in obtaining adequate control groups. For all of these reasons, most of the quantitative conclusions derived from human data are rather uncertain.

47. The embryonic or foetal age at irradiation is only rarely known precisely: the majority of the publications reviewed refer to irradiation performed during the first, second or third trimester of pregnancy, and in very few cases is the age of the sample under study specified by month. In addition, Nishimura *et al.* (226) pointed out a large difference between the developmental ages according to Streeter's horizon and the chronological ages estimated from ovulation in human embryos, showing a remarkable variation of morphological age for a given chronological age. It is therefore difficult to compare the stage sensitivity between man and other species on the basis of developmental scales such as that given in table 2, and this fact greatly limits the usefulness of the available observations. Dealing specifically with malformations, the most interesting period would be the second to the sixth week of the human embryonic life, because the induction rate of malformations is expected to be the highest on the basis of extrapolation from animal data. However, most human surveys refer to the last two trimesters or to the second half of pregnancy, where growth disturbances, rather than teratological defects, are the most common findings (74, 235).

48. The estimation of radiation dose in human investigations is also qualified by large uncertainties. In many cases the doses have been assessed indirectly. The earliest reports on children irradiated *in utero*, in the course of radiotherapeutical treatments of the mothers, are generally of clinical nature; furthermore, the importance of recording the physical parameters of the irradiation was then not fully recognized. Dekaban (71) examined over 200 literature cases of pelvic x irradiation of pregnant women and found that only about 10 per cent of them provided sufficient information (half-value layer, energy, distance, size of fields, current, irradiation time etc.) to allow crude estimation of doses. Even in the most recent and best planned epidemiological surveys (235), where the same apparatus and standardized techniques were involved and the average *per caput* dose in the exposed group was therefore accurately known, the individual doses showed a fairly large variation.

49. Most of the early Hiroshima and Nagasaki data were analyzed in terms of the distance of the irradiated persons from the hypocentre (371, 372) and their location with respect to various types of shielding materials (220). In addition, clinical symptoms of early radiation reaction shown by the mother helped to define the possible range of doses received by the child *in utero* (248, 371, 372). Critical re-evaluations of the doses received by the exposed individuals were performed at various times (8, 115), but only the most recent reports include reasonably satisfactory dose evaluations (196). Other methodological and technical problems encountered in the analysis of the human experience are discussed in other sections of this report.

J. THE MAIN PHASES OF PRE-NATAL DEVELOPMENT

50. The development of the conceptus in mammals is usually divided into three major phases: the pre-implantation phase, the period of major organogenesis and the phase of foetal 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 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 and invasion of the uterine epithelium by the trophoblast, may last 1-1.5 days in the mouse (266). In man the whole process may take a few days. A clear-cut separation between the embryonic and the foetal 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. Thus, the conventional limits of the three developmental periods, which are given for various species in table 3, are only rough approximations.

TABLE 3. APPROXIMATE TIME OF THE BEGINNING AND END OF THE MAJOR DEVELOPMENTAL PERIODS IN SOME MAMMALIAN SPECIES

(Days p.c.)

Species	*Pre-implantation*	*Major organogenesis*	*Foetal 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

Sources: Same as table 1.

51. The division in three phases mentioned above corresponds approximately to the most significant developmental events in the embryo and is also suitable for the description of radio-embryological effects, which are very different in nature and degree in the three periods (302, 303). For example, pre-natal death is characteristic of the pre-implantation phase (see chapter II) while neo-natal death and malformations are associated particularly with organogenesis (see chapter III). Irradiation during the foetal stage does not lead to gross malformations but rather to defects of growth, particularly in the central nervous system and gonads, or, at high doses, to post-natal death (see chapter IV). Carcinogenic effects following irradiation *in utero* are considered in Annex I.

52. This Annex deals in turn with the three periods of intra-uterine development. Within each period, it considers the effects observed in different animal species. Many of the experiments reviewed were actually designed to test the comparative effects of irradiation during different developmental stages. In general, these experiments will be described and discussed in the section dealing with the period when the most significant observations were actually made, in order to avoid unnecessary repetition.

II. THE PRE-IMPLANTATION PERIOD

A. LETHAL EFFECTS

1. Intra-uterine death

53. Russell and Russell (300) and Russell (295) have studied the lethal effects in pre-implantation mouse embryos after 200 rad administered each day between 0.5 and 4.5 days p.c. The embryos were observed at 10.5 and 13.5 days after mating, or 6 to 13 days post-irradiation. Embryos at the very early stages (0.5-2.5 days) were found, on the whole, to be more sensitive than those 3.5-4.5 days old. Death occurred long before the time of the first observation, so that any abnormality potentially present in these embryos could not have been recognized. Three modes of death were identified: *(a)* the death of entire litters before implantation, which was inferred from the excess of non-pregnant females over non-irradiated controls at the time of observation. This modality of death was estimated to account for about one third of all irradiated embryos; *(b)* individual deaths, also at the earliest pre-implantation stages, manifested by the decreased number of implants per pregnant female; *(c)* individual post-implantation deaths, assessed from the decreased percentage of living embryos in the irradiated samples.

54. Most of these conclusions were confirmed by the work of Rugh and Wohlfromm (286), giving rough estimates of the intra-uterine LD_{50} for embryos irradiated at various stages of pregnancy and observed at 18 days p.c. The greatest sensitivity was shown to occur at 0, 2 and 3 days p.c. with a relatively resistant phase on day 4 p.c. These early embryos, irradiated prior to implantation, rarely lasted sufficiently long to die as foetuses and were generally resorbed. The estimated LD_{50} for the pre-implantation stage is shown in table 4.

TABLE 4. ESTIMATES OF PRE-NATAL LD_{50} AND POST-NATAL $LD_{50/30}$ FOR IRRADIATION OF MICE AND RATS *IN UTERO*

	Mice					*Rats*					
Age (days p.c.)	*Pre-natal LD_{50} (rad)*				*Post-natal $LD_{50/30}$ (rad)*	*Pre-natal LD_{50} (rad)*			*Post-natal $LD_{50/30}$ (rad)*		
0		100									
1		350									
2		125									
3		140									
4	>200	330					30-120				
5		356									
6		380			400						
7		200			200						
8		165			155	140	120				
9	<200	225			140	150	120				
10		255	~150	80-160	120	215	160				
11	200-300	425	~200		160	250	210	>270			
12		560			175		240				
13		700			235						
14	>300	>700			275				>110		
15		>750			315						185
16		>800			365				110-220	214	
17		>800			500						
18					600				>220		
19											
20											
21										273	
Reference	295	286	68	217	289	362	27	324	212	253	322

55. A very careful analysis of the sensitivity of the mouse embryo to lethal damage during the first 48 hours of development was performed by Russell and Montgomery (299). Within the first 24 hours and with exposures of 100 R, very large variations of sensitivity were observed between developmental stages separated only by a few hours. With exposures of 200 R, the sensitivity, as shown by the pre-implantation loss, was found to be extremely high shortly after sperm entry and during the earlier pronuclear stages. At the two-cell stage it remained lowest to increase again at the beginning of second cleavage. Death occurred at random about the time of implantation and there were no late embryonic or foetal deaths.

56. Rugh and Grupp (278) and Rugh (263) studied the dose-effect relationship for embryonic death in CF1 mice irradiated at 0.5 or 1.5 days p.c., using five exposure levels ranging between 5 and 25 R. Under these conditions, the percentage of resorbed embryos showed

a regular increase at all doses in the 0.5-day series; in the 1.5-day series, the trend with dose was irregular but, on the average, the percentage was higher than in the controls (fig. I). Ohzu and Makino (232) performed a

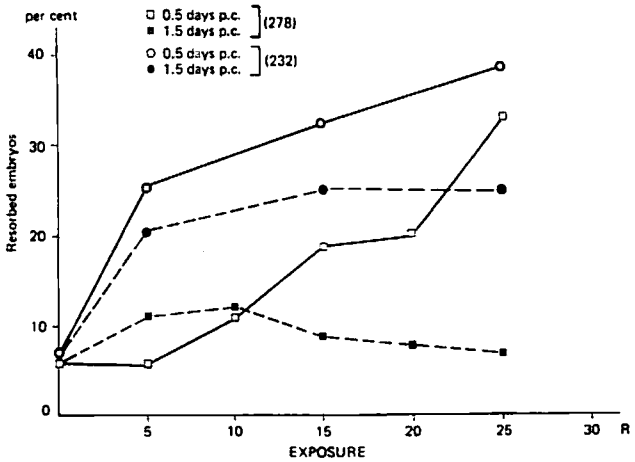

Figure I. Intra-uterine mortality after pre-implantation irradiation (mouse)

similar experiment on (dd X CBA)F_1 mice and also showed, for irradiation at 0.5 days p.c., an increase of the percentage of dead foetuses as a function of dose. Mortality values higher than control, with a plateau between 5 and 25 R, were observed at 1.5 days (fig. I). In a later publication, Ohzu (231) reported the same values for irradiation at 0.5 days, but different ones for irradiation at 1.5 days. Both series show exposure-related increases of the rate of resorption, but the author states that, although the increase of the rate of resorption is statistically significant in both the irradiated groups, the difference between the two groups is short of significance. If a linear regression analysis is carried out for the two sets of data obtained at 0.5 days p.c., the calculated average death increase is found to be 1.06 per cent per roentgen ($P<0.01$) for Rugh and Grupp's series (278) and 1.13 per cent per roentgen ($P<0.10$) for Ohzu and Makino's data (232). The two estimates are therefore in very good agreement.

57. Dose-effect relationships for embryonic death following pre-implantation irradiation were also reported in two recent publications. One publication (91) reports the results of experiments in which several doses of neutrons and x rays were given to pregnant mice, with embryos in the pronuclear zygote stage. Survival data were deduced from the examination of the conceptuses at 16 days p.c. A single exponential relation between the fraction of surviving embryos and absorbed dose was found by the authors to fit the experimental points closely up to a dose of 20 rad of neutrons or 100 rad of x rays. The values of D_0 (the dose necessary to reduce survival to e^{-1} or 37 per cent) were found to be 19 rad for neutrons and 87 rad for x rays. These doses would correspond to an average increase in mortality rate of the order of 0.8 per cent per rad in the x-ray series and of about 3 per cent per rad for the neutron series. The authors concluded that embryonic death could be considered a single-event process for both radiations and that the neutron RBE was about 4.5 (fig. II). The data can also be reasonably fitted by a linear relation, and the

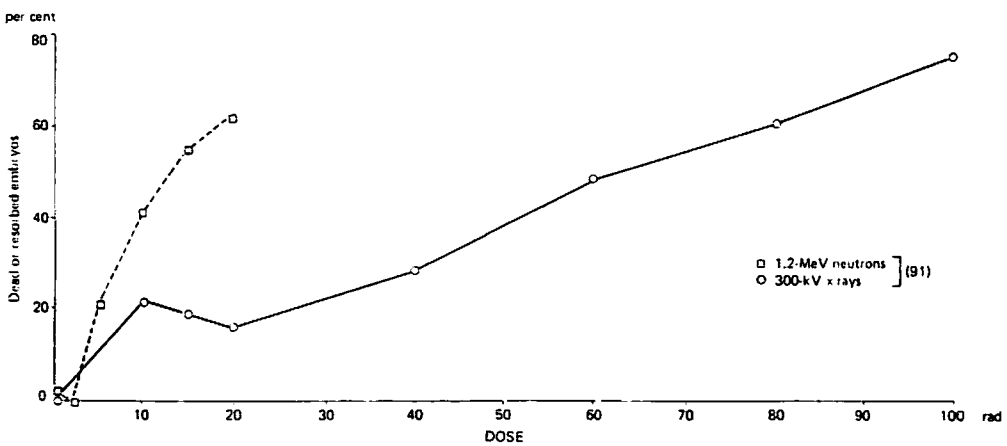

Figure II. Intra-uterine mortality after pre-implantation irradiation (mice exposed to neutrons and x rays)

dose to inactivate 50 per cent of the embryos would be, under this assumption, about 60 rad for x rays and 12 rad for neutrons. The agreement between these estimates of embryonic sensitivity to lethal damage and those discussed previously (278, 232) is indeed remarkable. Other results in the paper concerning the stage at which embryonic death takes place, are in accordance with results already discussed (see paragraph 53). Dose fractionation experiments, which were also reported in this paper, will be discussed in paragraph 311.

58. The other publication, by DuFrain and Casarett (77), reports the results of irradiation *in vitro* of zygotes from superovulated mice, the embryos having been cultured in microdrops of a chemically defined medium. The exposure required to inhibit 50 per cent of the embryos from reaching the two-cell stage was estimated to be about 760 R, and from reaching 50 per cent of the expanded blastocyst stage, about 130 R. As would be expected, the second value is higher than that estimated by Friedberg *et al.* (91) for the development of the embryo up to 16 days. This observation is also consistent with the well established fact that the majority of embryos die at cleavage but some can be lost after implantation has taken place. The *in vitro* culture technique also allows quantification of the cleavage delay observed after irradiation of the zygote. Other

data by Goldstein *et al.* (98) are reviewed in paragraph 69.

59. For the rat there appears to be conflicting evidence in regard to the death of entire litters. Some data show that such an effect may also occur in this species without signs of malformations after exposures of 400 R during the first week of development (123). On the other hand, other findings would question the presence of this phenomenon in the rat (33), though confirming a very high incidence of resorptions and an increased mortality of the implanted embryos after 150 R administered on the first day of pregnancy (32). There is agreement, however, that malformations are not seen in surviving conceptuses irradiated at the pre-implantation stages, even though the possible incidence of malformations in resorbed embryos remains, of course, unknown.

60. In hamsters exposed to 200 R of ^{60}Co gamma rays from the ovulation to the late pronuclear (110), and from the pronuclear to the early blastocyst. stages (0.5-3.5 days p.c. (111)). Harvey and Chang found an increase in pre-implantation lossess only when irradiation was performed at 0.5 day p.c. Post-implantation losses, on the contrary, were increased after irradiations at all times p.c. No malformations were observed. Other data concerning the irradiation of unfertilized ova of mice and hamsters and the subsequent development of embryos can be found in 173, 112, and 113. Female mongolian gerbils x-irradiated with various exposures from 200 to 1200 R on day 2 p.c. (two-cell stage of the fertilized egg) and examined on day 18 suffered severe pre-implantation loss down to 400 R but very few abnormalities were observed in the foetuses (189).

61. From the data of Chang *et al.* (59, 61) it can be concluded that the embryonic rabbit irradiated *in situ* before implantation (day 2-6 p.c.) with 400 R of gamma rays is very sensitive to radiation lethality. Irradiation of the fertilized ova *in vitro* followed by transplantation into recipient animals only resulted in increased mortality at exposures larger than 400 R. Surviving embryos were apparently normal and there was no evidence of differential sensitivity among the various stages of development such as that seen after irradiation *in utero.* In the work of Inman and Markivee (128) rabbit blastocysts of 3.5 days p.c. were exposed to 150 or 200 R of x rays and observed at 9.5 days of pregnancy. These exposures increased pre-implantation mortality from 7 to 18 per cent (average of both groups); post-implantation mortality contributed comparatively little to total mortality.

62. In the dog, irradiation with 150 R of ^{60}Co gamma rays at 5, 15 and 18 days p.c. (early blastocyst, late blastocyst and implantation stages, respectively) resulted in a total pre-natal mortality of 18, 24 and 48 per cent in the stages indicated, while the mortality for non-irradiated embryos was 18 per cent, showing that the dog embryo is most sensitive at implantation. At 8 days p.c., post-implantation mortality was low and it increased with increasing developmental age at which irradiation was carried out (245).

63. Thus, each species appears to have a period of high sensitivity to pre-implantation death in the very early stages. The mouse, rat and hamster are relatively resistant at implantation. The fact that the dog is sensitive at implantation could possibly be attributed to the late occurrence of implantation in this species, and coincides with the formation of the primitive streak, which is another period of high sensitivity in all species.

2. Post-natal death

64. Some data on neo-natal and post-natal death in animals irradiated at the pre-implantation stages are also available, mostly for the mouse. Rugh and Wohlfromm (289) reported that mouse embryos irradiated during the first 5 days of development with exposures from 125 to 400 R of x rays, had a heavy mortality *in utero;* the animals which survived and came to term had good survival during the first 30 days of post-natal life. In another experiment (275), mice irradiated at 0-5 days p.c. with previously determined (289) intra-uterine LD_{50} doses, showed less survival than the controls during the first 30 days of post-natal life. On the other hand, the long-term follow-up of animals surviving later than the first month showed an absence of life-span shortening, indicating that there was little permanent damage after the neo-natal period. Konermann *et al.* (157) performed an experiment in which mice were subjected to daily x-ray exposures of 40, 60 and 80 R during the main phases of pre-natal development. It was shown that irradiation during the period 1-5 days p.c. caused a high incidence of pre-natal death but had no effect (or little effect at the highest daily exposure) on the post-natal (3 weeks) survival of the offspring that were actually born. In dogs exposed to 100 R of x rays at day 13 and 16 p.c., neo-natal mortality increased from 34 per cent (control) to 45 and 57 per cent, respectively. A still higher mortality was observed, however, with irradiations at day 19 and 22 p.c., corresponding to the time of early organogenesis (245).

65. It appears, in conclusion, that pre-implantation death leading to implantation failure is the major mechanism of lethality in all species after irradiation at the early embryonic stages. Pre-implantation death may affect single embryos in polytocous animals or all the members of a litter. Intra-uterine death at later developmental stages is comparatively less important, and the survival of the animals which come to birth is practically unaffected by irradiation during the pre-implantation stages. There are quite substantial variations of the susceptibility to lethal damage, particularly during the early segmentation of the fertilized egg. The sensitivity at the time of implantation is usually lower than at the blastomeric stages, but differences between species can be observed. They may be explained by the different developmental organization reached by the embryos at the time of implantation and may not necessarily reflect intrinsic differences of the susceptibility of the different animal species. Dose-survival experiments in the mouse, in the range 5-100 R, show consistently that the increment in embryonic loss soon after fertilization may be of the order of 10^{-2} R^{-1}. The data on survival at different doses for early segmentation

stages are fitted well by simple linear or exponential relationships, which are not incompatible with relatively simple inactivation kinetics.

3. Mechanisms

66. Concerning the cause of pre-implantation death and the underlying mechanisms, Russell and Russell (302) reported that a high proportion of mouse embryos exposed to 200 R at second cleavage appeared to undergo a rapid disintegration of the nuclei, resulting in lethal damage, whereas irradiation outside cell division allowed further development of the conceptus, often up to implantation. Chromosomal damage in all cells derived from an injured blastomere gave rise to unbalanced chromosome complements. Such damage to the genetic material, together with large radiation sensitivity differences in the cell-division cycle, were confirmed for the first cleavage of mouse embryos (299). After irradiation, a high proportion of embryos showed subnuclei, suggesting that aneuploidy is responsible for most if not all, of the lethal damage. It was also shown that the state of the nucleus at the time of irradiation affects the production of aneuploidy, a fact which would account for the differences in sensitivity as a function of the cell cycle.

67. Rugh and Grupp (276, 277) reported results of extensive morphological studies of the damage induced by low doses of radiation in mouse embryos. Exposures as low as 5 R, at 0.5 or 1.5 days p.c., often resulted in fragmented embryos; the entire egg was broken up into globules, the further development of which appeared doubtful. Anomalies of cytoplasm and of nuclei, pycnosis and complete dissolution of the blastomeres were seen as early as 6 hours after irradiation (281, 282). Other histological studies, performed at 9 or 10 days p.c. on embryos irradiated at several pre- and post-implantation stages, showed that the damage, especially to the mesenchyme, was highest when embryos were irradiated early (310).

68. Kirkpatrick (149) followed the radiation induced morphological anomalies by means of *in vitro* cultures of mouse embryos during the two-cell stage. Also with this technique the irradiated embryos showed fragmentation, disintegration, granulation, nuclear degeneration and pycnosis, or cytoplasmic vacuolization. A distinct correlation was shown to exist between morphological abnormalities and embryonic death. The sensitivity of embryos irradiated *in vivo* or *in vitro* was found to be very similar, the sensitivity having been assessed from the dose required to stop the development of the embryo before the blastocyst in 50 per cent of cases (150, 77).

69. The experiments of Goldstein *et al.* (98) are similar in many respects to those just reported. They analyzed the radiosensitivity of pre-implantation mouse embryos by culturing them *in vitro* up to early post-implantation stages. X-irradiation was also performed *in vitro* at various doses when the embryos were at the 4-cell, 8-cell, morula and blastocyst stages, after which they were returned to culture and scored for the appearance of further events such as the formation of blastocyst, hatching, growth of the trophoblast or of the inner cell mass. Taking blastulation as the end-point, an increase in sensitivity was observed between the 2- and the 4-cell stage, with a decrease at the 8-cell stage. Radiation did not prevent blastulation when administered during the morula stage, but it impaired the occurrence of further events and the efficiency of this impairment was higher the further removed the specific end-point was from the irradiation stage. Growth of the inner cell mass appeared on the whole as the most sensitive indicator of the damage. Up to the 8-cell stage pre- and post-implantation death was responsible for developmental failure, whereas irradiation at the morula and blastocyst stages resulted predominantly in post-implantation lethality. In spite of minor deviations, these findings appear to be in fair agreement with the other data *in vitro* reviewed previously (150, 77) and also with *in vivo* experiments (91, 299, 278, 232). All these data contribute to strengthen our knowledge of sensitivity and mechanisms of damage of the early mammalian embryo.

70. The relationships between the damage to single blastomeres and death of the whole embryo have also been studied. On the assumption that the probability of killing a blastomere is independent of the number of blastomeres in the early pre-implantation embryo, Russell (295) calculated that embryos surviving irradiation at these stages of development included probably some in which one or more blastomeres were injured. Since virtually all surviving embryos appeared to be normal, a considerable degree of totipotency of the blastomeres was inferred. On the other hand, it seems logical to believe that the proportion of damaged blastomeres over the total number present at the various developmental stages, together with their capacity for regulation, may influence the survival of the embryo. It may be of interest in this context that recovery from lethal x-ray damage (300 or 500 R) of the embryonic rat during hormonally induced delayed implantation has been observed by Ward *et al.* (355).

B. DISTURBANCES OF GROWTH

71. The data available on growth disturbances induced by pre-implantation irradiation of mice refer mostly to post-natal observations. Stunting has been reported in the mouse during pre-natal growth (276, 277), but it seems difficult to envisage the existence of a growth disturbance unconnected with other effects, such as lethality or malformations.

72. Regarding post-natal life, an exposure of 100 R of x rays at 0-5 days p.c. did not produce loss of weight or reduction of skeletal size within 4 months of age, while the same exposure at later pregnancy stages produced appreciable stunting (271). Pre-implantation mouse embryos subjected to LD_{50} doses of x rays and which survived to an age of 24 months showed no gross adverse effects on weight. In some instances, the animals with an *in utero* radiation history were even heavier than controls, probably because of the reduced average litter size. These mice also showed no permanent defect of skeletal growth, as determined by radiographs of selected bones performed at the age of 2 months (275).

The offspring at term of mothers exposed daily to 40 to 80 R during the pre-implantation period did not show any loss of weight with respect to controls, but actually showed a significant post-natal overgrowth (157).

73. Brent and Bolden (32) and Brent (31) reported that in the rat the growth of the surviving embryo exposed to 150 R during pre-implantation was not retarded, but this finding contradicted the data of Skreb *et al.* (326), who showed a statistically significant loss of weight in rats irradiated at 5 days p.c. and examined at 15 days of pregnancy. Rabbits irradiated with 150 or 250 R at 3.5 days p.c., and weighed at 9.5 days, showed a reduction of body size observable in spite of the variability of body size among controls, since lighter foetuses in the controls were not as numerous or severely affected as in the irradiated groups (128).

74. It may be concluded that reduction of body growth due to irradiation during the pre-implantation period is not an effect so clearly and universally recognized as embryonic death, even at exposures in excess of 100 R.

C. MALFORMATIONS

75. The appearance of malformations following irradiation of early pre-implantation embryos has been reported in a few instances. Such malformations involve particularly the central nervous system and the skeletal system. In the mouse, a maximum of 6 per cent exencephaly was produced (277) by irradiation with 50 or 200 R of x rays during pre-cleavage, when no organ primordia but only the three primary germ layers are present in an embryo. In another report (278) the dose-effect relationship for this abnormality was examined in embryos of 0.5 or 1.5 days p.c. within the range 5-25 R. The occurrence of the cerebral malformation was confirmed, but no obvious trend with dose was established. The authors believe that damage of the sensitive neuroblasts, which is the cause of exencephaly during organogenesis (see paragraphs 135-140), is not the only possible mechanism of production of this malformation. At early stages of development (gastrula), irradiation could interfere with the cellular movements in a way that alters the process of cephalization of the embryo, causing the appearance of abnormalities of the head structures.

76. The presence of a few cases of exencephaly was also conformed by Ohzu and Makino (232) and Ohzu (231). These authors irradiated fertilized mouse eggs at 0.5 and 1.5 days p.c. and reported, in addition to exencephaly, an average incidence of forelimb polydactilia of 28 per cent and 21 per cent, respectively. The incidence seemed to be unrelated to exposure in the interval 5-25 R. Hind-foot digits were practically unaffected. Since forelimb polydactilia was also observed in 14 per cent of the non-irradiated mice, it was concluded that the increased incidence after irradiation could be indirectly due to an effect on the uterine environment (subsequent, for example, to heavy lethality of the pre-implantation embryos) rather than to a direct radiation effect on the blastocysts. It should be mentioned that exencephaly also occurs in normal mice (in about 1.5 per cent of the embryos of the CD-1 strain), and that irradiation during the mononuclear-zygote stage (91) does not consistently modify its incidence. Also, chromosomal imbalance may frequently lead to exencephaly in the mouse. Therefore, the relationships between an aberrant cytogenic constitution and the appearance of gross malformations could be investigated in greater depth to establish precise pathogenetic mechanisms.

77. Brent and Bolden (32, 33) reported data for embryo rats exposed on the first day of gestation to 150 R, with various combinations of ovary, oviduct and uterine shielding. They were unable to find any malformation in more than 1,000 offspring examined. A small number of irradiated rabbit blastocysts gave rise to embryos that were severely stunted and grossly abnormal in body proportions or retarded in differentiation 9.5 days p.c. The degree of the malformations was such that these animals would probably not have survived long enough to be scored as malformed at birth (128).

78. In the dog (245), developmental malformations were usually not seen following pre-implantation exposure, and the only two cases of severe cranio-facial abnormality seen after an exposure of 150 R at 15 days p.c. were attributed to uncertainty of the foetal age at irradiation.

79. In conclusion, the few reported malformations in mouse foetuses irradiated during the pre-implantation stages (notably exencephaly) represent a very improbable event, particularly in comparison with the frequent occurrence of aberrations during organogenesis. These malformations can be explained embryologically, and their occurrence does not contradict the general view that the death of the embryo, rather than the induction of malformation, is by far the highest risk of irradiation during the pre-implantation stages.

D. OTHER EFFECTS

80. A variety of other effects have been reported, mainly in the mouse, as a result of *in utero* irradiation during the pre-implantation stages. They are mentioned in this report for the sake of completeness but are clearly too scanty for meaningful generalizations. The sex ratio of mice irradiated *in utero* with x rays at different times and doses (289, 292) was equal to that of controls. Similarly, there was no shift of this ratio in the animals dying during the first 30 days post-partum. These observations show that radiation is not selective with respect to sex in inducing mortality.

81. Rugh, Wohlfromm *et al.* (292) reported a significant increase of the incidence of gross lens opacities (sclerosis of the nucleus and overt cataract) particularly in male mice irradiated with 100 R of x rays during the fertilized pre-cleavage stage and followed up to 18 months of age; however this increase was smaller than some values reported earlier (270, 275). It was suggested that the pathogenesis of the radiation-induced cataract might be related to a damage of the developmental processes that was insufficient to be

lethal. Another effect observed in these mice after irradiation prior to implantation and survival for up to two years, was a slight increase in the white-cell count and a small reduction in the red-cell count (275).

82. Data on post-natal fertility of the male mouse x-irradiated at various gestational ages from conception to 18.5 days p.c. were also reported, showing that exposures up to 100 R had little effect (288).

83. Sex-chromosome losses were studied in the progeny of mice irradiated within 48 hours after copulation. Marked changes of sensitivity to this effect were shown to occur within a few hours, in parallel with similar oscillations of sensitivity to lethal damage (299).

III. THE PERIOD OF MAJOR ORGANOGENESIS

A. LETHAL EFFECTS

1. Failure of pregnancy

84. The reduction in the percentage of copulations that result in pregnancy due to irradiation at various stages of pre-natal development is a measure of mortality involving the complete loss of a litter. This indicator is rarely mentioned in the recent literature but was used in the earlier papers (see Russell (295) for a review). In mice, Russell (295) has shown that complete interruption of pregnancy, which is the frequent result of pre-implantation exposure (see paragraph 53), is a comparatively rare event after post-implantation irradiations. As an example, exposures up to 400 R between 9.5 and 13.5 days p.c., when pregnancy may be diagnosed externally, result in almost 100 per cent litter-bearing females, while an exposure of 200 R, between 0.5 and 8.5 days p.c., when pregnancy is not readily apparent, clearly decreases that yield. From these data it also appears that this indicator of lethality is not sensitive for the case of exposures in the post-implantation stages. Such a conclusion is supported by the observations of Jacobsen (136), who noted the variability of the estimates and the absence of effects with exposures of 100 R at 7.5 and 10.5 days p.c.

2. Litter size

85. The decrease in litter size at or just before birth reflects the death of individuals in a litter due to mechanisms which could in principle operate before or after implantation. Jacobsen (136) pointed out that, although this indicator is defined as the ratio between the number of offspring and the number of mothers, its exact specification depends on the choice of which mothers are considered for the ratio: mothers with one or more offspring at the observation time, all pregnant females including those aborting and dead, or mothers that completed pregnancy, including those having offspring or resorptions. With each choice, the indicator has a different biological meaning, but, nevertheless, in all cases essentially similar dose-effect relationships are found.

86. The change in the average litter size has been studied as a function both of developmental age at the time of irradiation (fixed dose) and of dose. This indicator is an easy one to measure and relevant data may be found in many papers. Only some of them are reviewed here. Rugh, Duhamel *et al.* (270) have reported in CF1 mice the values of litter size at birth after exposure to 100 R at different times p.c. The data show (fig. III) a reduction in litter size of the order of 40 per cent with irradiation soon after copulation. For irradiations at a later time, a stabilization of the indicator at about 10 per cent below normal is found, but a further decrease occurs at around 7 days p.c., followed by a return, with oscillations, towards control values. The observations reported by Dekaban (72), obtained by exposing Webster Swiss mice to 200 R at different post-implantation times, are qualitatively similar (fig. III). The average litter size is about 30 per cent of control for irradiation at 7 days p.c.; after irradiation at later times, the indicator reaches the control value by day 12 p.c. and remains unchanged until day 18 p.c. These data consistently show that the

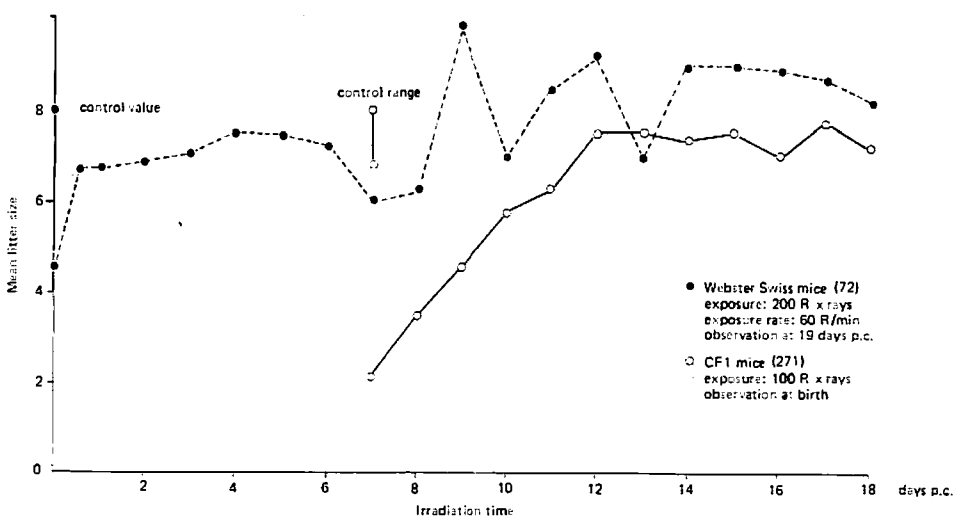

Figure III. Effect of irradiation at various p.c. times on mouse litter size

sensitivity to radiation lethality, as measured by litter size, is maximal immediately after conception and during the early stages of organogenesis and decreases at later times to low and constant values during the foetal stages of development.

87. In the rat, early data by Job *et al.* (139) and more recent data by Murphree and Pace (212) show that exposures of 110 to 220 R in the period 13 to 20 days p.c. have no effect on litter size at birth. The slight changes in litter size in the dog reported by Phemister *et al.* (245) are of doubtful significance with an exposure of 100 R at 16 and 19 days p.c.

88. An analysis of the available data on litter-size reduction as a function of dose shows that different dose-effect relationships are valid in the different embryonal and foetal stages examined. The data of Russell (295) obtained on embryos in the pre-implantation, post-implantation and foetal stages are shown in figure IV. For irradiations in the period 0.5-4.5 days

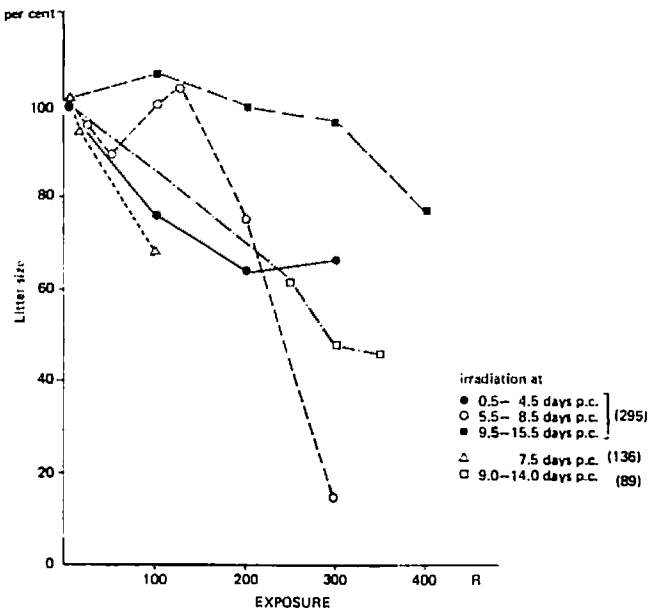

Figure IV. Dose-effect relationships for mouse litter size at birth after irradiation at various p.c. times

p.c., a monetonically decreasing relation is found up to exposures of about 200 R, followed possibly by a constant tail. For irradiation during organogenesis, there seems to be an absence of effect with exposures up to 125 R: at higher exposures, the effect increases substantially with exposure. The relationship for irradiation during the foetal stages show much smaller effects in the same dose range.

89. The data of Fraser and Hall (89), obtained by irradiation during the embryonal and early foetal ages, show a much more important decrease in the litter size at the tested exposures (fig. IV) but are of little help for establishing the form of the relation since they cover only the interval between 250 and 350 R. The low-exposure region is adequately covered by Jacobsen's observations (136), which show an apparently linear exposure-response curve between 5 and 100 R in 7.5-day-old embryos (fig. IV); however, Jacobsen did not consider this effect as a particularly sensitive indicator of lethality in his mouse strain. There is a discrepancy between the linearity of the response found by Jacobsen and the shoulder-type relationships obtained by Russell (295) and by Kriegel *et al.* (160), although the stages of pregnancy at irradiation and the exposure conditions in the two experiments seem to have been comparable. Nash (217) has also studied the litter size at birth in mice of various genetic backgrounds, with four different x-ray exposures (20, 80, 160 and 320 R) at four development ages (6.5, 10.5, 14.5, and 17.5 days p.c.) (fig. V). There appears to be

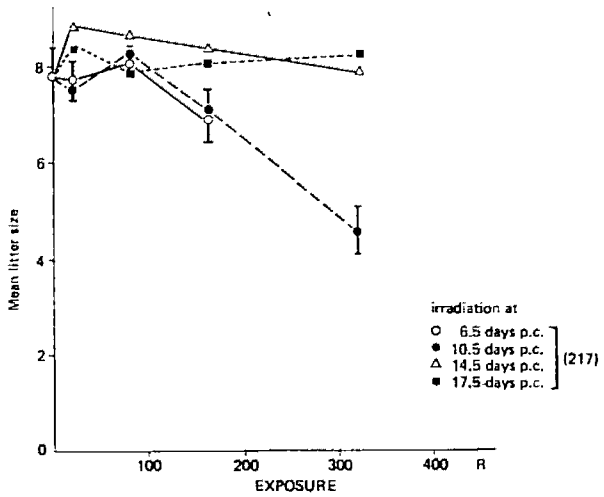

Figure V. Dose-effect relationships for mouse litter size at birth after irradiation at various p.c. times

little or no effect at all ages up to 160 R, and the only point significantly different from the control is that at 320 R in the 10.5 days p.c. series. It should be mentioned, however, that in this series all animals were dead soon after birth.

3. Resorptions

90. Special attention should be paid to the phenomenon of pregnancy resorption, which is rather commonly described in experiments with rodents and observed even during normal pregnancy in a sizeable but variable percentage. As shown by Jacobsen (136) in the mouse, the incidence of resorptions increases drastically with the age of the mother and is very sensitive to seasonal influences and to weak extrinsic teratogenic stimuli, including radiation. Jacobsen (136) broadly classifies the resorption types observed in the mouse. His classification may not be sufficient for an accurate description of mechanisms, but it does make possible broad evaluations of the stage of pregnancy in which resorptions could have occurred. On the basis of the work of Kameyama (142) and his own observations, Jacobsen (136) considers resorption to be a very sensitive indicator in the case of radiation exposure, provided that the conditions under which it is scored are rigorously specified and the strain characteristic and extrinsic factors are properly controlled or allowed for.

91. Since implantation is a prerequisite of resorption, a radiation-induced change in resorption frequency should essentially reflect post-implantation mortality.

There are data on this effect in the mouse covering various days p.c. around the time of implantation or various doses at fixed gestation times. Murakami and Kameyama (204) have reported no change of embryonic loss in mice with exposures up to 50 R at 8 days p.c.; with exposures of 100 R, the fractions of resorbed and missing embyros were about 20 and 30 per cent, respectively, while the control loss was less than 10 per cent. Kriegel *et al.* (160) exposed pregnant mice to 200 R of x rays at different times between 5 and 15 days p.c. and scored resorptions on day 18 p.c. Under those conditions, they were able to show (fig. VI) that

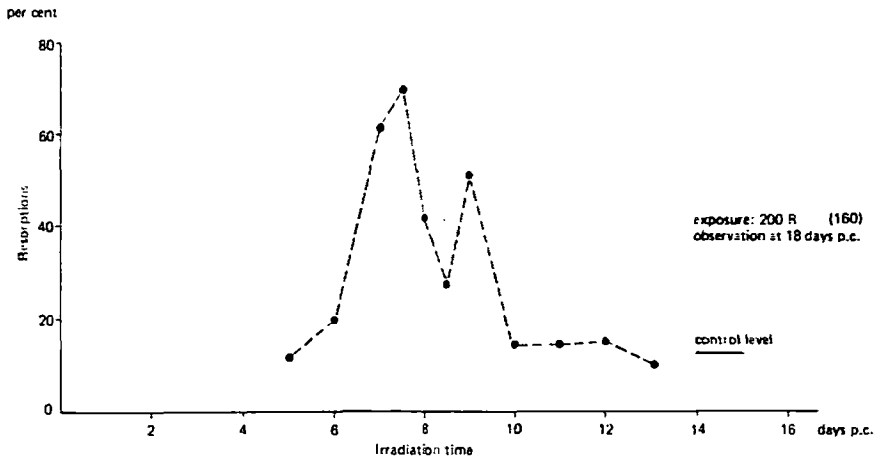

Figure VI. Effect of irradiation at various p.c. times upon resorption incidence in the mouse

the incidence of resorptions, which was around control level (12 per cent) with irradiation at 5 days p.c., increased substantially with developmental age at irradiation, reaching a peak of 70 per cent at 7.5 days p.c.; for older ages at irradiation, the incidence decreased to the control level at about day 10 p.c.. The embryonal lethality was also examined as a function of exposure in

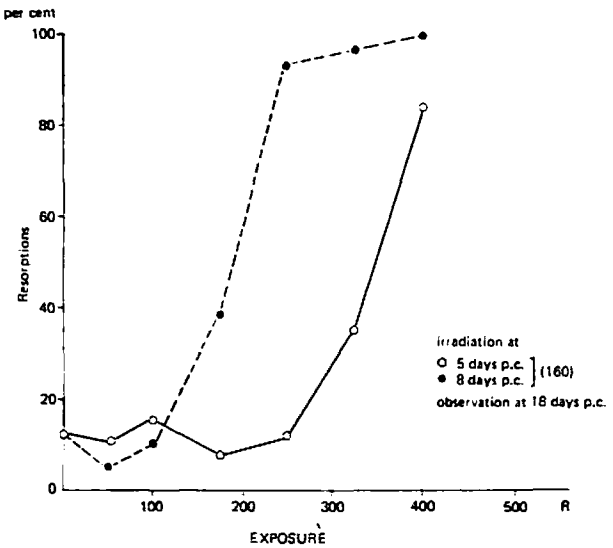

Figure VII. Dose-effect relationships for resorption incidence in the mouse

the range of 50 to 400 R, with two irradiation ages (5 and 8 days p.c.). The curves (fig. VII) show marked differences in that they begin to rise steeply at widely different doses. being fairly parallel from then on; the maximum effect occurs between 250 and 400 R at 5 days p.c. and between 100 and 250 rad at 8 days p.c.

92. These data (160) are at variance with the apparently linear dose-effect relationship between 5 and 100 R reported by Jacobsen (136) for a different strain of mice (fig. VIII), but under comparable experimental

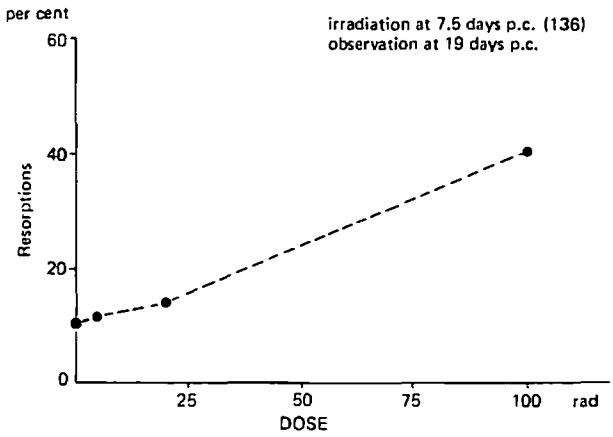

Figure VIII. Dose-effect relationship for resorption incidence in the mouse

conditions (irradiation at 7.5 days p.c., scoring at 19 days p.c.) Even allowing for differences in the technique, the fact that in Jacobsen's experiment (136) an exposure of 100 R increased the incidence of resorptions from the control value of 10 per cent to 40 per cent shows that there is a large variability of sensitivity to radiation among animal strains. Moreover, the different shape of the dose-effect curves (fig. VII) supports the existence of a variation of sensitivity between embryonal stages, as shown already by the timing experiments previously discussed (160).

93. The data of Skreb and Bijelić (325) in the rat (fig. IX) establish the existence of a stage-differential sensitivity to the induction of resorption, with a maximum at 8.5 days, in good agreement with the

mouse data. The work of Skalko (324) with animals of 11 days p.c. exposed to 135 or 270 R confirms that at this gestational age the resorption effects are weakly correlated to the level of exposure.

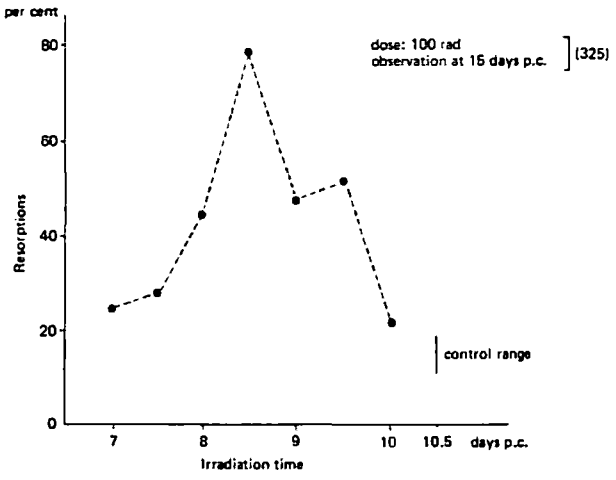

Figure IX. Effect of irradiation at different p.c. times on resorption incidence in the rat

4. Mortality *in utero*

94. The percentage of embryos alive before term is the most straightforward and sensitive indicator for evaluating the lethal action of radiation on single embryos and foetuses. It measures the induced global impairment of the complex sequential process that leads to a fully viable individual. Dagg (68) irradiated inbred strains of mice *in utero* (fig. X) with exposures in the

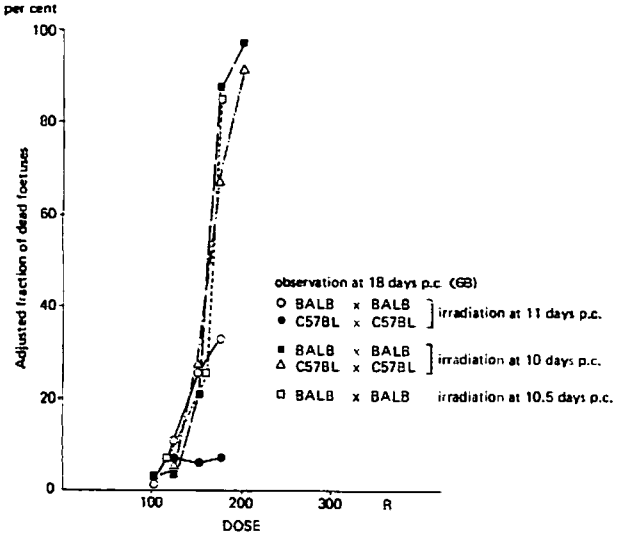

Figure X. Dose-effect relationships for mortality *in utero* for various inbred mouse strains

range of 100 to 200 R and at times between 10 and 11 days p.c. and scored live and dead offspring at 18 days p.c. With irradiation at 11 days p.c., the incidence of dead embryos in the BALB/C strain increased with exposure in the range of 100 to 175 R, for the C57BL strain there was little indication of such a response. However, when irradiation took place at the same developmental age in both strains, both survival curves became similar, even though the BALB/C mice showed a somewhat higher sensitivity. For both strains there was a threshold for the lethal effect of the order of 140 R. The mouse strain 129, on the other hand, was highly resistant to exposures up to 200 R; differences were also seen in hybrid crosses between these inbred strains.

95. Wilson (362) irradiated Wistar rats of 8 to 11 days p.c. with exposures in the range of 12.5 to 600 R. In this experiment (fig. XI) 8-day-old embryos were apparently

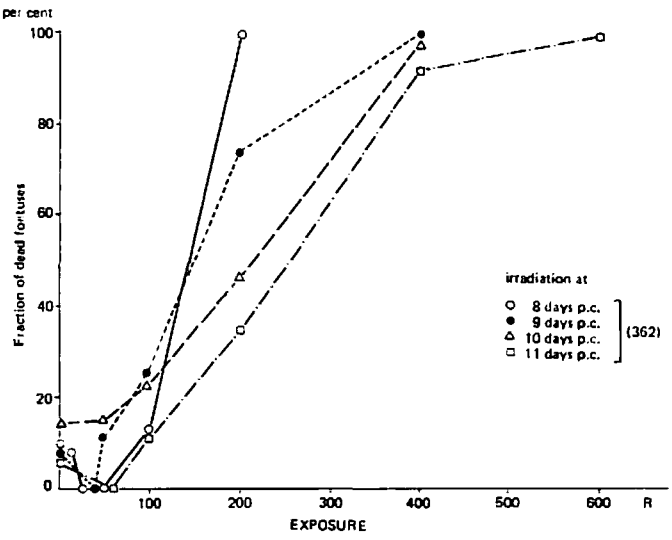

Figure XI. Dose-effect relationships for intra-uterine mortality in the rat

resistant up to 100 R but reached 100 per cent mortality at 200 R. At increasing embryonic age, the sensitivity to radiation lethality tended to decrease, giving rise to mortality curves that were less steep.

96. In the hamster, an exposure of 200 R during pre-implantation causes a moderately high mortality. The same exposure at implantation results in low mortality and at 6.5 days p.c. (time of primitive streak formation), in the highest mortality. At older developmental ages, mortality gradually decreases to the control value during organogenesis and foetal growth (fig. XII). At the time of peak sensitivity, an exposure of only 100 R induces about 70 per cent degenerating foetuses, the 100-per-cent effect being produced by 200 R (111, 59). The data for the rabbit reported by the same authors (59) show that mortality following an exposure of 400 R is 100 per cent if irradiation takes place at the time of implantation and tends to fall if irradiation is carried out later during organogenesis and foetogenesis. There is also a fairly complete experimental series with beagle dogs exposed to 150 R (245), in which pre- and post-implantation mortality has been studied as a function of irradiation time from 8 to 35 days p.c. The results show that post-implantation and total mortality are significantly higher than control values (fig. XIII) for irradiations at a gestational age of 18 to 22 days p.c.

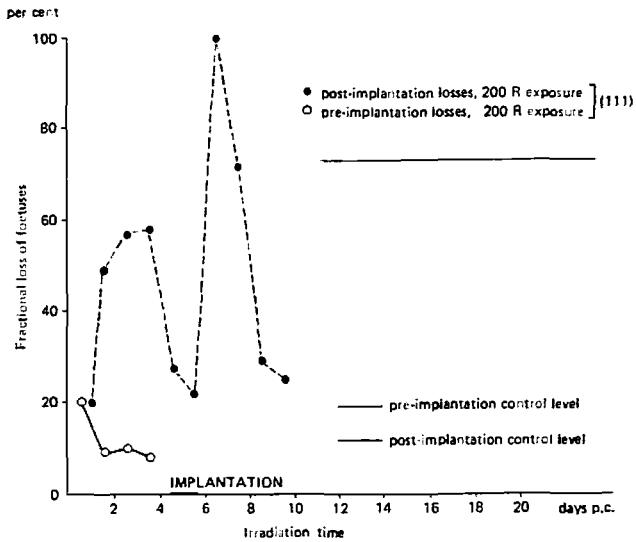

Figure XII. Effect of irradiation at various p.c. times on intra-uterine mortality in the hamster

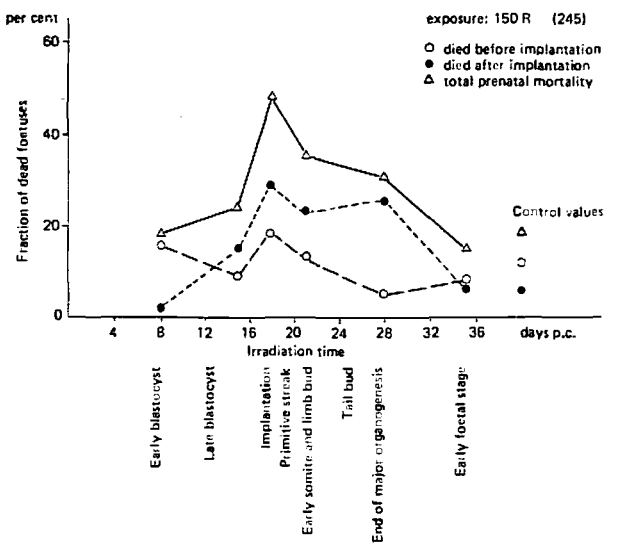

Figure XIII. Effect of irradiation at various p.c. times on intra-uterine mortality in the dog

(during the phases of implantation, primitive streak and early somite formation), in good agreement with the data for rabbits.

97. In conclusion, it appears that pre-natal mortality in many species is highest for irradiations after implantation and during the stages of early organogenesis, and that subsequently mortality decreases with the advancement of embryonic differentiation. From the available data in rats and mice it would appear that the shape of the dose-lethality relationship for *in utero* mortality might change substantially with developmental stage and that, in the large majority of cases, at all post-implantation ages tested a sigmoid relationship would apply with an increase in the slope occurring in the region of a few tens of rads. At the later stages of pregnancy, the decrease of sensitivity is mainly reflected by lower slopes of the mortality curves. There is insufficient information to identify the possible mechanisms of foetal death at these early post-implantation stages.

5. Neo-natal mortality

98. Russell (295) reported data on neo-natal mortality in mice from which it is quite apparent that exposures of 100 R or lower have no effect on survival at birth; 200 R induce a peak of mortality when received between 7.5 and 11.5 days p.c. (early organogenesis); 300 R at the same times induces 100-per-cent mortality, the effect falling off steeply with irradiation at times up to 14.5 days, in a manner apparently parallel to the 200 R curve. It can be concluded that around 50-per-cent mortality, the slope of the curve is very steep for any given developmental stage.

99. In a more recent report (217) on mice of three different genetic strains and their crosses, the pooled data shows that 20 or 80 R at any age between 6.5 and 17.5 days p.c. does not cause any neo-natal mortality, while 160 R causes a peak mortality of up to 75 per cent at 10.5 days p.c., and 320 R results in a mortality of 100 per cent when received at 10.5 days p.c.. 23 per cent at 14.5 days p.c. and zero at 17.5 days p.c. These data are in very good agreement with those cited previously. Neo-natal mortality in beagle dog puppies exposed to 100 R of x rays during a period from just before to just afer implantation is highest for irradiations at 19 and 22 days p.c., corresponding to early organogenesis (fig. XIV).

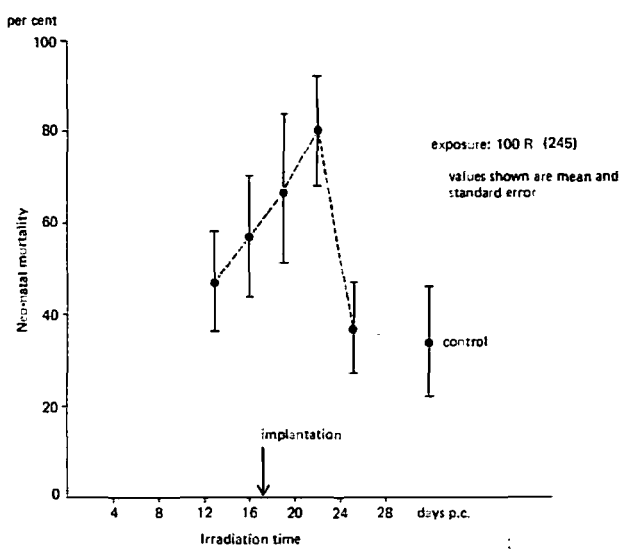

Figure XIV. Effect of irradiation at various p.c. times on neonatal mortality in the dog

100. In conclusion, therefore, at exposures below about 100 R there is no (or little) increase of neo-natal mortality, but the effect becomes manifest at higher exposures, with an increasing slope of the dose-effect relationship. As differentiation proceeds, the sensitivity of the embryo and foetus gradually decreases.

101. There is information available for two rodent species on the LD_{50} for irradiation *in utero* and on the variation during embryonic and foetal development shown in table 4. The variability among species and strains found in different experiments is very large, the identification of general trends being correspondingly difficult. It appears, however, that sensitivity is very high (with LD_{50} corresponding to exposures lower than

150 R) in the pre-implantation, implantation stages and early organogenesis stages. In the single case where timing was accurate, the fine structure of the radiosensitivity of the mouse embryo showed considerable variability, but a large share of this variation could be attributed to the technical conditions under which the observations were made rather than to intrinsic changes in the sensitivity of the embryo. LD_{50} data for the mortality *in utero* reported for the period between day 6 and 13 range from 165 to 700 rad. At the late stages of organogenesis, the LD_{50} begins to rise gradually to reach during foetal development values comparable to the post-natal LD_{50} levels.

6. Post-natal and long-term mortality

102. The mortality after birth of animals irradiated *in utero* was studied by Rugh and Wohlfromm (289), who irradiated CF1 mice at 6-18 days p.c. to establish the radiation dose that would kill, within 30 days after birth, 50 per cent of the mice born alive. The $LD_{50/30}$ values (table 4) were found to fluctuate with developmental age. The lowest values were at 8-10 days p.c., the time when the most active differentiation of the nervous system takes place. On days 6, 7 and 8 p.c., the LD_{50} *in utero* (286) was the same as the $LD_{50/30}$, which implies that exposures of about 400, 200 and 150 R at these ages, respectively, kill half of the offspring *in utero* and half of those born alive within 30 days. At later exposure times (see table 4) the $LD_{50/30}$ were consistently and substantially lower than the LD_{50} *in utero*, showing that, while the embryo cannot easily be killed before term, it can readily be damaged in a way which renders it unable to survive the first month after birth. Early post-natal deaths were more frequent among litters irradiated during early gestational ages.

103. The observations of Nash (217) are in general agreement with these data, in spite of minor technical differences. He reported that the survival to 60 days of mice still alive 12 hours after birth was maximum after irradiation at 6.5 days p.c. Irradiation at 10.5 days p.c. had the most severe consequences, while exposures at 14.5 or 17.5 days p.c. produced intermediate effects. For example, after 80 R at 10.5 days p.c., only 55 per cent of the mice alive soon after birth were still surviving at 60 days, while 62 and 78 per cent survived if the same exposure occurred at 14.5 or 17.5 days p.c., respectively. No consistent pattern of survival differences between sexes, doses or embryological stages was observed. Inbred mice appeared to survive less than hybrids, especially immediately after birth, and in the groups where the increase in post-natal mortality was most significant, inbred strains showed a larger response to the radiation insult than hybrid genotypes.

104. An unexplained and still not confirmed conditioning effect has been reported by Rugh and Wolff (293), whereby an exposure of 10 R of x radiation on day 15.5 p.c. enabled mouse foetuses to survive better when irradiated as adults with doses around the $LD_{50/30}$. This "beneficial" effect was also apparent at 14.5 and 16.5 days p.c., while higher exposures (25 to 300 R) led invariably to deleterious effects in adult life.

105. The data of Nash and Gowen (219) extended the observations to the whole life span of mice, showing that the reduction in longevity after 20 to 320 R at four gestational ages depended on genetic constitution, sex, gestational age at irradiation and exposure. The influence of these factors was evaluated in multifactorial experiments in which data on mature life spans of 647 animals were collected.

106. In the rat, the neo-natal death rate was significantly higher than control (6 per cent) when the litters were exposed to 110 R on day 13-15 p.c. (27 per cent) and on day 15-18 p.c. (13 per cent), to 150 R on day 14 p.c. (98 per cent), and to 220 R on day 12-16 p.c. (77 per cent) (118). Sikov, Resta and Lofstrom (322) carried out an analysis of the long-term mortality of rats surviving at weaning, with exposures of 20 or 100 R at 10 days p.c. and of 50 or 185 R at 15 days p.c. Sex differences were also found in this study; life-span shortening in females appeared to be greater than in males, in which the effects observed were of doubtful significance and in any case not very substantial.

107. It can be concluded that the lethal effects of irradiating animals *in utero* are not exhausted with pre-natal mortality but at appropriate doses may also become manifest in post-natal life. Thus, a dose of radiation insufficient to kill an animal *in utero* may still be lethal after birth. Post-natal $LD_{50/30}$ values calculated on live-born animals in the region of 150-300 rad have been reported for the rat and mouse irradiated between day 6 and 13 of intra-uterine life. Mortality after birth takes place essentially during the first month after birth. Long-term reductions in life span may also become evident for exposures in excess of 200 R in rodents, depending on a number of genetically controlled factors of the strain. A detailed discussion of tumour induction in animals irradiated *in utero* will be found in Annex I.

108. In the human species there are no data related with certainty to the lethal effects of irradiation during embryonic stages. Since there are great difficulties in tracing the pregnancy stage at irradiation in most of the reported experiments and since most of the experience refers to irradiation in the foetal stage, this subject will be reviewed in connection with the irradiation of the foetus (paras. 192-195).

B. DISTURBANCES OF GROWTH

1. Intra-uterine growth

109. Russell (294) reported foetal growth defects in mice irradiated between 0.5 to 13.5 days p.c. with exposures of 200, 300 and 400 R. The mean weight at birth of these animals was not significantly below control for irradiation times up to 7.5 days p.c. The effect was considerable with irradiations at later stages of development. The response curves as a function of irradiation time for the three exposures were found to be roughly parallel, with maximum effects between 10.5 and 11.5 days p.c. A complete and apparently linear dose-effect curve for heterozygous but genetically

uniform animals irradiated at 11.5 days p.c. was reported by Russell, Russell and Major (305) covering the interval 0-300 rad. The resulting average weight reduction was estimated to be 0.22 g per 100 rad within this dose range, in fair agreement with the previously reported data. Jacobsen (136), irradiating homozygous HC mice at 7.5 days p.c. (a relatively less sensitive stage, according to the data of Russell (294)), reported a reduction of 0.12 g per 100 R, for animals sacrificed at 19 days p.c.

110. In view of the experimental differences between the two sets of data it is difficult to draw a clear conclusion, but it seems possible to assume that weight reduction is a more sensitive indicator of radiation damage if irradiation occurs at 10.5 and 11.5 days p.c. rather than earlier or later in foetal life. Considerable strain variations are, however, to be expected (136, 266). This view is also supported by more recent work by Konermann (155), who studied experimentally the relative sensitivity of the mouse conceptus to irradiation during blastogenesis, organogenesis and foetal stages, with fractionated exposures of 10 to 80 R per day. Animals examined 18 days p.c. appeared to be most sensitive when irradiated during organogenesis. When irradiation continued during the foetal growth, a still higher effect was produced, with weight reductions up to 50 per cent at exposures of 80 R per day.

111. In a study of radiation effects on embryonic growth, Yoshizawa and Ueda (373) irradiated dd-Y mice *in utero* at various gestational ages (7-17 days p.c.) and at various exposure levels (0-300 R) and exposure rates (1-100 R/min) of a ^{137}Cs gamma source. The body weight of the foetuses and the growth of the caudal vertebrae were taken as indicators of the effects to be studied; they appeared to show similar dependencies on the experimental variables. The gestational ages 8-11 and 16 days p.c. were found to be sensitive stages for the inhibition of bone growth, and they appeared to be related to inhibition of the cartilage bud and to damage of the ossification processes, respectively. The delay in the degree of ossification and the decrease in body weight were insensitive to exposure rate in the interval indicated. However, both effects were clearly enhanced by increasing exposure. In this case (irradiation at 11 days and sacrifice at 18 days p.c.) the weight reduction was of the order of 0.23 g/100 R, in good agreement with the data above (para. 109).

112. In the rat, Wilson (362) examined growth retardation after exposures of 12.5-200 R at days 8, 9, 10, 11 p.c. The effect was assessed as weight loss at various times after irradiation, according to a fairly complete experimental schedule. The degree of retardation tended clearly to increase with increasing exposure (although not in direct proportion) at all embryonal ages tested. However, the exposure to obtain a given effect was larger in older embryos, showing that the embryo becomes increasingly resistant to growth retardation. In fact, the weight retardation effect per 100 rad may be estimated to be 14-30 per cent (depending on the observation day) of the control in the 8-day embryo, about 17 per cent in the 9-day embryo, 6-14 per cent in the 10-day embryo and about 2 per cent in embryos aged 11 days.

113. More recent data have added little to these general conclusions. Campi *et al.* (58) made similar observations after 100 rad of x rays given 10 days p.c. and showed that the longitudinal and transversal embryonic diameters, as well as the weight of the foetuses, were considerably reduced 7 days later. Skreb *et al.* (326) exposed embryos between 5 and 10 days p.c. to 100 R of 85-kVp x rays. In reasonable agreement with the data of Wilson (362), the weight of the embryos on day 15 was up to 30 per cent smaller than control, particularly if irradiation took place 8.5-9 days p.c., during the onset of mesoderm formation.

114. It is difficult however to draw more quantitative estimates from experiments where the sacrifice was carried out, not at a fixed time or at maximum weight loss, but at variable times post-irradiation. Weight reductions of 7 per cent after 135 R and 28 per cent after 270 R were reported by Skalko (324) on rats irradiated at 11 days p.c. and observed at term. Brent and Bolden (32) obtained a weight reduction of 11 to 16 per cent at term after 150 R at 6 days p.c., but were unable to show any effect at the same exposure given on the first day p.c. Growth retardation was also reported in mongolian gerbils given 200-400 R of x rays at the primitive streak stage (189). In the rabbit, irradiated at 3.5 days p.c. and observed 6 days later, 150 or 200 R significantly reduced the size of the embryo, in a manner roughly proportional to exposure (128).

115. The data reviewed show that in several species the period of major organogenesis is particularly sensitive to radiation-induced growth disturbances, possibly more than the foetal period but particularly more than the preceding blastogenesis phase. The size of this indicator varies with the time at which the dose is administered during organogenesis and with the interval between irradiation and observation. In all species there are insufficient data to evaluate the net effect of a dose at a fixed post-irradiation time or at the time giving maximum expression of the damage.

2. Post-natal growth

116. Radiation-induced growth disturbances may persist during post-natal life. Levy *et al.* (171) have reported significant reductions of the dimensions of the femoral, parietal and mandibular bones of the mouse, following an exposure of 300 R of x rays at 15.5 days p.c. This effect lasted at least up to 240 days of age, when the last samples were taken. Rugh, Duhamel *et al.* (271) made a complete skeletal study of animals exposed to 100 R *in utero* at different times of gestation (0-18 days p.c.) and which were alive at the age of 4 months. They showed that, both in male and female offspring, stunting in all bones examined was more pronounced if irradiation had taken place between 11 and 13 days p.c., the time when the embryo undergoes the earliest skeletal differentiation. Additionally, a significant reduction of body weight occurred in males irradiated on day 5 p.c. and on day 9-18 p.c., and in females irradiated on day 8-18 p.c., the most drastic reduction being the result of irradiation on day 11-14 p.c. This shows that the second half of gestation is the most significant period for radiation-induced

stunting. Stunting persists during the whole life, since similar results are obtained if the average weight between 3 and 35 months of age is taken as the indicator instead of single measurements at fixed times (272).

117. In other experiments by Nash and Gowen (218), three genetically different strains of mice were exposed to 20-320 R at different times between 6.5 to 17.5 days p.c., and then their post-natal growth was followed for a period up to 75 days of age. The observed body weight reductions were found to depend on dose, gestational age and post-natal age. Reduction was maximum for mice irradiated at 10.5 days p.c. and minimum for those irradiated at 6.5 days p.c., the other two embryological ages tested (14.5 and 17.5 days p.c.) giving an intermediate response. Weight reduction reached its maximal expression when the animals were about 40 days old and remained constant afterwards. There was a marked difference in response between strains, which could be attributed to the difference in the capacity for recovery from the radiation insult and also to variations in developmental age at the time of irradiation.

118. Results available for the rat lead essentially to the same conclusions. Alexandrovskaya (2) observed that 16-month-old rats which received a dose of 150-200 rad on day 12 p.c. had pronounced hypoplasia of the central nervous system. Murphree and Pace (212) reported that rats exposed to 110-220 R at various gestational ages from 13 to 20 days p.c. had average weaning weights lower than the controls. The weights of the body and of the testes in adult males which had been irradiated were also significantly lower than control, and the testicular weight depression was dependent on dose and age at irradiation. Sikov, Resta and Lofstrom (322) showed that an exposure of 20 or 100 R at 10 days p.c. and similarly an exposure of 50 or 185 R at 15 days p.c. reduced the weight at birth in all groups, except at the lowest dose. Growth depression persisting to older ages up to about 80 weeks was only evident in groups irradiated at 15 days p.c., and this effect was manifest also for single organs, particularly for the brain and the spleen, with differences between sexes. It seems likely that the reduction of body and organ weights, often associated with histopathological changes and depression of hormonal levels, cannot be entirely explained by stunting, being probably also the result of complex physiopathological deficiencies.

119. Significant reductions of the interzygomatic distance and of the skull length were observed in adult dogs exposed *in utero* to ^{60}Co irradiation during the organogenesis and foetal stages but not during the pre-implantation period (167). A linear dose-effect relationship seemed to apply between 20 and 330 R, and the fractional reduction per 100 R was estimated to be 4-5 per cent for the interzygomatic distance and 1.7-2.5 per cent for the skull length. Irradiation at the foetal stages was slightly more efficient (167). The volume of the eye in these animals was also smaller than in controls (168).

120. Growth disturbances observed in humans irradiated in the embryonic stages will be discussed in paragraphs 202-208, together with the description of growth deficiencies following foetal exposure. This form of presentation is consistent with the fact that growth changes (as opposed to malformations) are the most likely outcome of irradiation during the foetal stages.

C. MALFORMATIONS

1. General

121. Some qualifying considerations are necessary for the analysis of malformations produced by irradiation during the major organogenesis period. A major difficulty is the lack of standardized nomenclature of malformations, each author having followed his own criteria for classification. This difficulty is compounded by the fact that malformations of adjacent but embryologically unrelated structures are often found in the same animals, complicating substantially the systematic treatment of the data.

122. As most of the published work was performed for the purpose of embryological research, it is very hard to extract from these publications the information relevant to the study of radiation effects. Sufficiently complete analyses of dose-effect relationships for various malformations have been performed in only few cases; most observations have been made at a single or very few doses. In addition, if one considers the variability of species and strain, the very different criteria under which the malformations have been scored and the fact that experiments differed in irradiation conditions, it is not surprising that the various experimental series can hardly be correlated.

123. The Committee believes, nevertheless, that some general conclusions can be derived from consideration of such material. The main emphasis is obviously on reported data that could be used for assessing dose-effect relationships, and for that reason the following review should be regarded as selective and by no means complete.

2. External head abnormalities

124. External head abnormalities described in connection with embryonic irradiation comprise: *(a)* abnormal shapes of the head (dome-shaped or vaulted cranium; narrow, long head); *(b)* abnormalities of nostrils and the snout (tapered, beak-like); *(c)* abnormal shape of the maxilla, jaw and mouth; *(d)* cleft lip or harelip and cleft palate and face; and *(e)* abnormalities of the external ear. Cranial blisters are described below in subsection 4, malformations of the central nervous system (paras. 135-140), and microcephaly is treated in a special section (subsection 5, paras. 141-146) in view of its importance in human radiation biology.

125. In the mouse, the maximum sensitivity for inducing malformations with exposures of about 200 R occurs between 7.5 and 10.5 days p.c. (294) or between 7.5 and 13.5 days p.c. (72), although each malformation probably recognizes a specific "critical period" with smaller exposures (209). According to Jacobsen (136), exposures in the range 5-100 R at 7.5 days p.c. induce few malformations of the head, jaw and mouth. These

results are in accordance with those of Nash (217), showing that vaulted cranium is induced only at exposures of at least 160 R (at 10.5 days p.c.) and also with the observations of Dagg (68), where cleft palate occurred only at exposures of at least 100 R (at 11 or 10.5 days p.c.). Murakami *et al.* (209) found abnormally shaped heads, harelips and cleft palates following exposures of 200 R on day 9 p.c. The same authors (210) reported malformations of snout, maxilla, mandible, harelip and cleft palate following exposures of 150 to 300 R in the period 7-12 days p.c. It appears that in the mouse 50 R is the lowest exposure at which occasional abnormalities of this type are found (204).

126. Studies on the morphogenesis of the palate and on mechanisms of cleft palate induction after 300 R of x rays administered to two strains of mice at 11 days p.c. were reported by Callas (56) and by Callas and Walker (57). The progress in palate development was evaluated by morphological rating in relation to the chronological age and to the embryonic weight. These studies showed that the primary cause of the malformation could be traced to a delay of the movement of the palatine shelf during embryogenesis. The A/Jax mouse appeared to be more susceptible than the C57BL to the induction of this malformation.

127. Warkany and Schraffenberger (357) described malformations in the rat, following exposures up to 1120 R at 9-15 days p.c. These malformations included defects of the skull with partial protrusion of the brain, cleft palate and lip, and maxillary and jaw abnormalities. Their incidence depended markedly on exposure and time of irradiation, but the data are insufficient for an analysis of the dose-effect relationships. More recently, Rajtova and Horak (251) reported malformations of the chrondrocranium in animals exposed to 200 R at 12-15 days p.c. The maximum effects were seen with irradiation at 13 days p.c.; they included degenerative processes and defects of the nasal wall and septum, of the turbinate primordia and of the lamina cribrosa. The incidence of malformations observed with irradiation at 15 days p.c. was not greatly different than in controls.

128. Cleft palate and cleft lip were also found in hamsters (200 R on day 7.5 p.c. (111)), in monogolian gerbils (200-400 R on day 9 p.c. (189)) and in rabbits (400 R on days 9-11 p.c. (59, 61)). Severe cranio-facial malformations, harelip and cleft palate were observed in dogs exposed to 100-150 R immediately preceding or following implantation. They were frequently accompanied by major abnormalities of the brain and eyes (245). Cleft palate and micrognatia were also seen in monkeys following 250 R of x rays during major organogenesis (249).

3. Malformations of the eye

129. Russell (294) reported malformations of the eye in mice exposed to 200-400 R at 7.5-10.5 days p.c. They included various degrees of microphthalmia including anophthalmia, coloboma of different types, and narrowing of the pigmented ring of the iris. Eyelids open at birth is also a pathological condition in this species. Russell's data are insufficient for establishing relevant dose-effect relationships. Anophthalmia and microphthalmia were also seen by Murakami and Kameyama (204) in mice exposed on the day 8 p.c. to 100-150 R, but not if exposed to 25 or 50 R. Majima (181) was the first to report a dose-effect relationship for gross eye malformations, applicable to irradiation at 8.5 days p.c. (fig. XV). This relationship had a threshold of 25 R, in

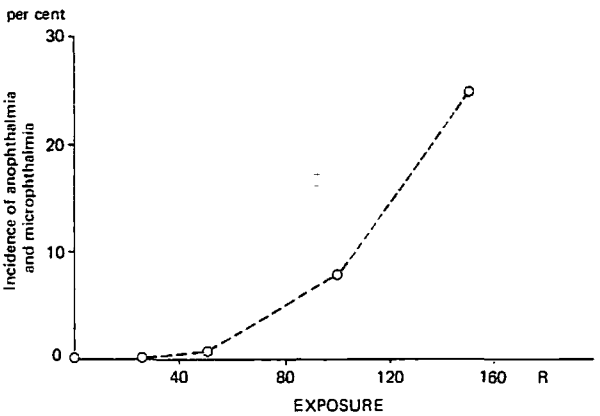

Figure XV. Dose-effect relationship for induction of anophthalmia and microphthalmia in the mouse (181)

the sense that no increase of abnormalities was seen at this dose. The defects were invariably associated with malformations of the central nervous system, and the author described the histopathogenic changes responsible for the malformations. In a second paper (182), Majima irradiated mice 8.5 days p.c. with a fixed dose of 200 R and studied the embryos by gross and microscopical observations at invervals of 1 day between 11 and 19 days p.c. Histology showed the presence of microscopic changes a few days after irradiation.

130. About 29 per cent of eye malformations in ddN mice were reported by Murakami *et al.* (209) following exposure to 200 R at 9.5 days of pregnancy. Murakami *et al.* (210), in their report dealing specifically with malformations of the extremities, include also data on the incidence of microphthalmia and anophthalmia in ddN mice exposed to 150, 200 and 300 R on day 7-11 p.c. From these data it is very difficult to identify the period of maximum sensitivity. Murakami (203) found differences between ddN and CFl mice in this critical period for the induction of eye abnormalities, using exposures of 200 and 300 R (fig. XVI).

131. Sakurai (307) confirmed interstrain differences and the presence of two sensitivity peaks in one of the two mouse lines examined. In addition, he studied microscopic morphogenetic changes at various intervals within 24 hours of irradiation. Experiments on eye malformations in mice were also reported by Badtke *et al.* (250) (130 R at various p.c. times between 7 and 11 days), showing a sensitivity peak between 7 and 8 days, and by Degenhardt and Franz (70) (140 R on day 6-11 p.c.), who obtained essentially the same type of information on the same mouse strain. Other data on eye abnormalities in the mouse are also to be found in papers by Jacobsen (136) and Nash (217), where exposures of 5-100 R did not have clear effects.

132. The most recent and complete set of data on the histopathology of radiation-induced eye malformations

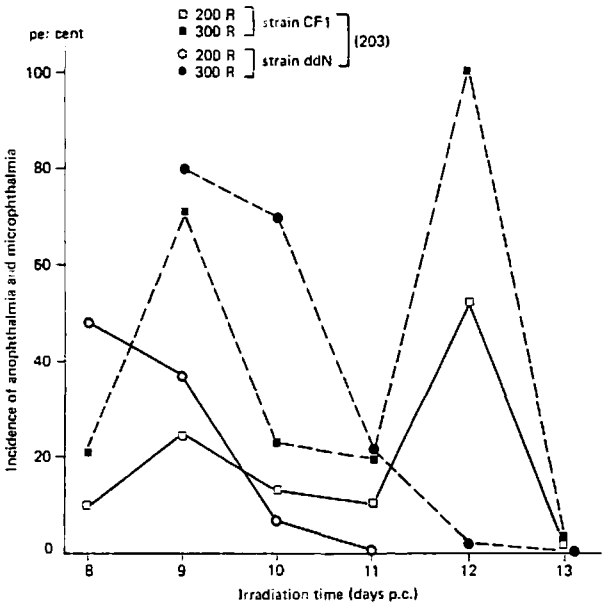

Figure XVI. Effect of irradiation at various p.c. times on the incidence of gross ocular malformations in the mouse

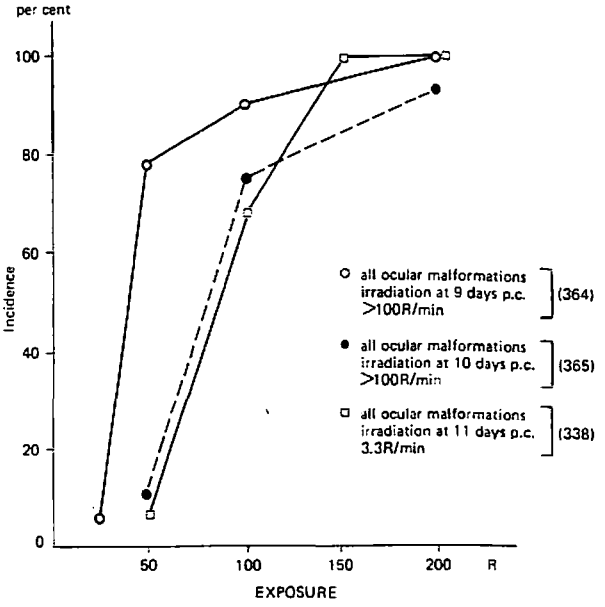

Figure XVII. Dose-effect relationships for the induction of gross ocular malformations in the rat

in the mouse is that of Christensen (65, 66). He performed extensive investigations on the C3H mouse between 7 and 14 days p.c. at exposures of 55 to 255 R. Animals were examined before birth and gross, stereomicroscopical and histological changes were recorded accurately. The amount of data provided is very large in respect to many effects, but, limiting the present comments to eye malformations, the maximum response was found at 8-11 days p.c. with 222 R. By far the greatest number of malformations were observed after this dose and only a few after 55 and 166 R. Some well defined effects (coloboma, microphthalmia and mild palpebral defects) were found to be stage-specific. Since the study was meant especially as a histopathological one, with a particular view to stage-specificity, the data on dose-effect relationships are not as informative as they might have been otherwise. However the data showed that severe microscopic changes were present in eyes that would be classified as normal on gross or stereomicroscopical examination. Other histological effects in various mammalian species were also reported by Rugh and Skaredoff (284), Lucas (174) and Hicks *et al.* (122), as well as in many of the previously cited papers on malformations of the eye.

133. In the rat, the eye has been reported as the most consistently affected of all organs. Anophthalmia, microphthalmia and other malformations are found following exposure at 9 or 10 days p.c. (362, 364, 365). Dose-effect relationships (in the range 25-200 R) have been deduced for irradiation at these times. Even the lowest exposure produced about 5 per cent ocular malformations if delivered at 9 days p.c. (fig. XVII) (364, 365). It appears that, with exposures up to 100 R, microphthalmia has the highest incidence, while for 200 R anophthalmia prevails. A 59-per-cent incidence of anophthalmia was reported by Skalko (324) in animals exposed to 270 R of ^{60}Co radiation at 11 days p.c. Strange and Murphree (338) have examined the exposure-rate dependence of eye malformations in this species (11 days p.c.), providing reasonably complete exposure-incidence curves in the range of 50-200 R at dose-rates of 1, 3.3, and 47 R/min; 3.3 R/min appeared to be the most effective exposure-rate (see also paragraph 318) and produced at 50 R a 7-per-cent incidence of animals with defective eyes (fig. XVII). These curves might be described as roughly curvilinear.

134. Microphthalmia, anophthalmia and other malformations of the eye were induced in hamsters. The incidence was 38 per cent with exposures of 100 R at 6.5 days p.c., 52 per cent with 200 R at 7.5 days p.c., and 65 per cent with 200 R at 8.5 days p.c. (111). Eye, brain and visceral malformations were seen in rabbits exposed to 130 or 250 R of x rays on the 10th day of pregnancy (353). Finally, ocular abnormalities have also been reported in dogs following exposures of 100-150 R before or after implantation (13-38 days p.c.) (245).

4. Malformations of the central nervous system

135. The gross malformations of the central nervous system (CNS) resulting from irradiation of embryos comprise exencephaly (or pseudencephaly), anencephaly, encephalocele, hydrocephaly (dysgenetic hydrocephaly) and spinal cord abnormalities (flexion, hernia, hydromyelia, myeloschisis). They are often accompanied by other malformations of the bones and structures of the head. The "cranial blisters" that some authors describe as skeletal abnormalities are in fact small brain hernias.

136. In mouse experiments with 150-200 R, the peak incidence of exencephaly is observed with irradiation at 8 to 9 days p.c. (fig. XVIII) (276, 277, 210, 72), while the sensitive period for hydrocephaly extends to 10 days p.c. (fig. XIX) (210, 72).

137. A detailed classification of the abnormalities of the CNS was published by Kameyama (142) and by Murakami and Kameyama (204). The mice were irradiated on day 8 p.c. and examined on day 13, and the x-ray exposures were in the range 25-150 R. Occasional

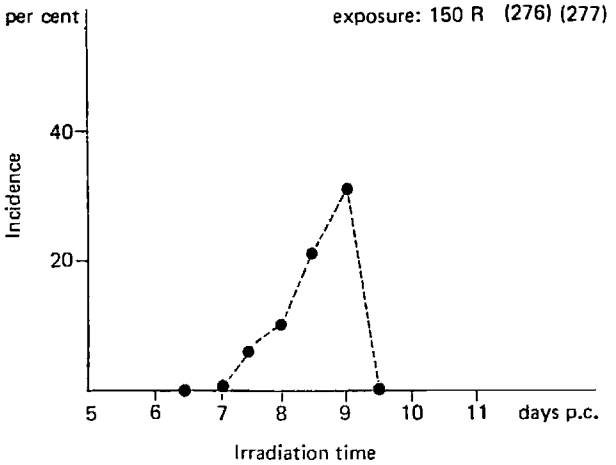

Figure XVIII. Effect of irradiation at various p.c. times on induction of exencephaly in the mouse

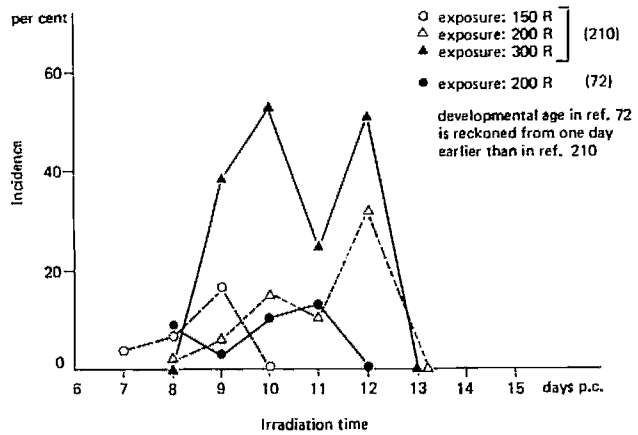

Figure XIX. Effect of irradiation at various p.c. times on induction of hydrocephaly in the mouse

malformations (exencephaly, hydrocephaly, flexion of the spinal cord) were observed at exposures as low as 25 R, the incidence increasing progressively at higher exposures. Exposure-effect data for exencephaly are reported also in two papers by Rugh and Grupp (276, 277) for the exposure range 25-200 R. Pooling together all abnormalities seen at each exposure level in the four groups of mice irradiated between 7.5 and 9 days p.c., it can be shown that the incidence is very low at exposures smaller than 100 R, while at exposures of 150 and 200 R the incidence is 17 per cent and 16 per cent, respectively. Murakami (203) and Murakami *et al.* (206) observed the incidence of exencephaly and hydrocephaly in mice of two strains exposed to 200 or 300 R at 8-13 days p.c. The observations were at 19 days p.c., and showed some difference between the strains. The same authors also described the histopathogenesis of these malformations in animals sacrificed serially with the purpose of correlating the malformations with the developmental events of the embryonic nervous system at the time of irradiation. Histopathological observations have also been reported by several authors (204, 276, 23, 72, 73, 237, 238, 239, 116, 126). Irradiating on day 7.5 p.c., Jacobsen (136) did not find cases of exencephaly with exposures of 20 and 100 R, although a few cases did appear in the control groups.

138. Wilson (362) summarized the observations on abnormalities of the CNS in rats reported by Wilson and Karr (365) and Wilson *et al.* (364, 363). The rat brain appears to be affected differently depending on time of irradiation, between 9 and 11 days p.c. On day 9 p.c., several brain malformations were produced, resulting from the fusion of the brain wall with the ectoderm. On day 10 p.c., the effects were hypoplasia and growth irregularities of various parts of the forebrain, while on day 11, eversion of the choroid plexus and other minor distortions were induced. From the few data relating dose and malformation incidence quantitatively, it would appear that exposures as low as 50 R on day 9 p.c. result in an observable incidence increase. With irradiation at older ages p.c., embryo CNS-abnormalities appear only with higher exposures. Skalko (324) reported an incidence of 81 per cent of hydrocephaly following an exposure of 270 R on day 11 p.c.

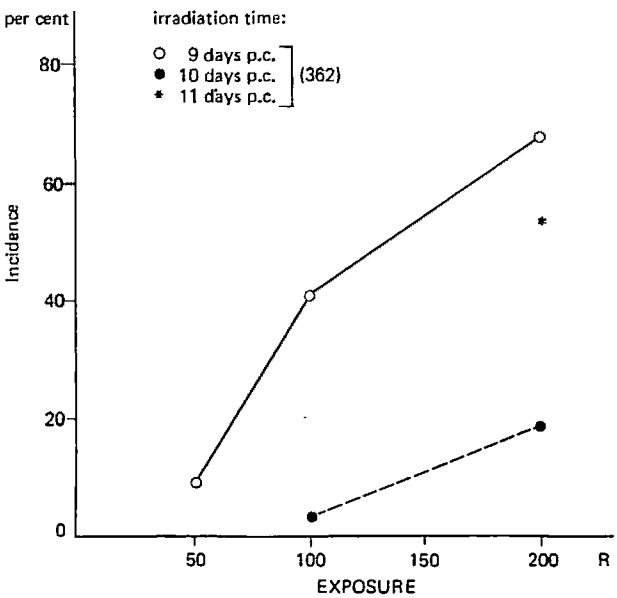

Figure XX. Dose-effect relationships for the induction of brain malformations in the rat

139. In reference 121 Hicks and D'Amato summarized their own previous work (118, 119, 122) and results from other sources. The summarized work related mainly to CNS malformations and correlates malformations with embryological stage rather than with radiation dose. Histopathological studies show that abnormalities of the microscopic architecture of the brain are seen in general at rather low doses. Riggs *et al.* (257) and Hicks and D'Amato (120) have reported results on various alterations of the nerve cell and of the cerebral cortex: cortex alterations can be induced by exposures as low as 10 to 40 R. It may be said, in conclusion, that although more observations have been carried out in rats than in mice, they are usually insufficient for dose-effect studies and their importance lies mainly in the establishment of correlations between time of irradiation and type of resulting malformation, owing to the differentiation of the relevant structures at the time of irradiation.

140. In hamsters, exencephaly was seen at exposures of 200 R on day 6.5 and 7.5 p.c. (111). In dogs, exposures of 100 to 150 R on day 13-38 p.c. caused CNS malformations, which were reported in a single class with ocular defects by Phemister *et al.* (245). Marmosets irradiated with 250 R of x rays during organogenesis developed cranioschisis (249).

5. Microcephaly

141. In the mouse, the peak of sensitivity for inducing microcephaly with 200 R is at day 10-11 p.c. (210, 206, 72); in the rat, on the other hand, it is at day 12-13 p.c. (206) (fig. XXI). Very few dose-response data are available for the mouse. Murakami *et al.* (210) report for

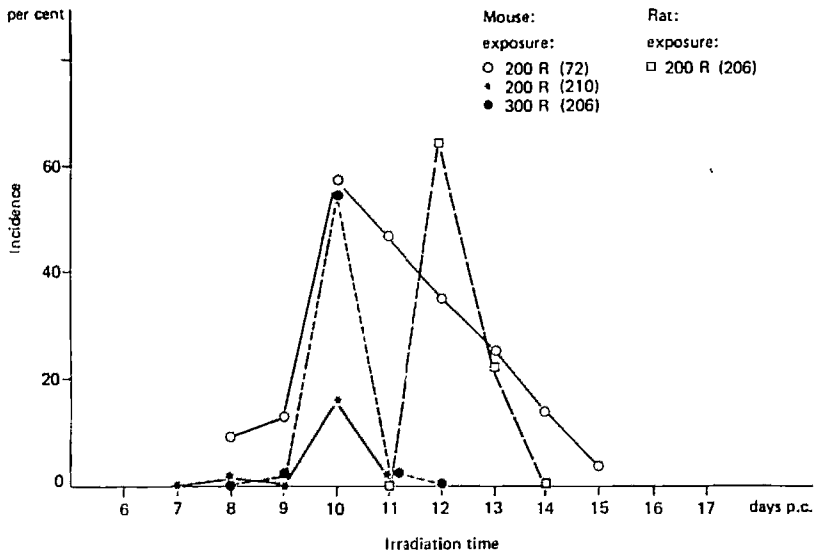

Figure XXI. Effect of irradiation at various p.c. times on the induction of microcephaly in the mouse and rat

irradiation at peak sensitivity no incidence of microcephaly with 150 R, about 15 per cent with 200 R and about 17 per cent with 300 R. In another publication (206), the incidence with 300 R is reported to be about 55 per cent, an incidence similar to Dekaban's estimate (72) of 55 per cent for exposures around 200 R. It is justified to assume that the technique of scoring the malformation may have influenced the estimates in these cases, since the head diameter is a continuous variable. It is doubtful (206) that the microcephalic condition in the mouse (which is accompanied by abnormalities in morphogenesis) could be compared to the "genuine" microcephaly seen in man. Ballardin and Metalli (13) have exposed (C57BL X C3H)F_1 mice to 50-200 R on day 9 and 16 p.c. The net weight of the brain was estimated post-natally at the age of 3 months. A statistically non-significant decrease of 1.5 per cent of the brain mass was shown following 50 R on day 9 p.c., while 100 R on day 16 p.c. resulted in a very significant decrease, amounting to 9.6 per cent in the male, and 7.5 per cent in the female, offspring.

142. The induction of malformations in the CNS of the human embryo (particularly microcephaly with associated mental retardation) has been the object of several reviews (95, 96, 97, 121, 213, 294, 370). These reviews considered the earlier work, which consisted mainly of isolated observations, mostly of a qualitative nature. More recently, Dekaban in his 1968 review (71) and the Committee in its 1969 report have analyzed in detail the whole subject of CNS abnormalities in man (345).

143. Dekaban (71), studying more than 200 literature cases of therapeutic pelvic irradiation of pregnant women, selected 26 cases as being sufficiently informative and estimated the probable doses received by the embryos. Having established that in no case was the exposure of the embryo lower than 250 R, Dekaban attempted to correlate the type of damage observed with the gestational age at irradiation (from about 2 to about 25 weeks). The most frequently observed abnormalities in this series were these: small size at birth and stunted post-natal growth; microcephaly, often associated with mental retardation; microphthalmia; pigmentary degeneration of the retina; genital and skeletal malformations; and cataract. Dekaban was able to conclude that incidence in man followed patterns similar to those established in experimental animals, and that the presence and type of abnormalities depended upon the gestational age at irradiation. Irradiation during the pre-implantation stages or at times not exceeding 4 weeks of gestation can lead to high lethality, but the children that survive are likely to develop normally. Between about 4 and 11 weeks many organs and systems appear to be sensitive to irradiation, as shown by the variety of malformations produced. The brain appears to be one of the organs most consistently affected, particularly with production of microcephaly. At 12-16 weeks, stunted growth and microcephaly is still induced, but gross abnormalities of other organs are not seen. Finally, microcephaly, mental retardation and stunted growth are induced, in a mild form, with irradiation at 16-19 weeks; irradiation in the period 20-25 weeks causes no obvious abnormalities but only minor defects of haemopoiesis and of the skin. It should be pointed out that large doses can be lethal to the embryo at any gestational age.

144. In the review carried out by the Committee in its 1969 report (345), a rough estimation is given of the

possible incidence of mental retardation associated with microcephaly. The value is about 10^{-3} rad^{-1}, derived from observations at high acute doses (in excess of 50 rad). The report stressed the uncertainties involved in extrapolating these findings to smaller doses, due to the absence of suitable human data and to insufficient knowledge of the mechanisms responsible for the production of malformations.

145. A recent report by Miller and Blot (196) considered the dose-effect relationships for microcephaly on the basis of the Hiroshima and Nagasaki experience and updated dose estimates. A progressive increase of the incidence with dose was observed in children exposed *in utero* before the 18th week of gestation and examined at 10 years of age (388 children in Hiroshima and 99 in Nagasaki). The Hiroshima data, which were more abundant, revealed a higher frequency and severity of the condition when exposure occurred within 3 to 17 weeks of gestation, but some effect was present with irradiation at older stages. While the lowest effective total kerma in Hiroshima was between 10 and 19 rad, in Nagasaki no effect was found up to a total kerma of 150 rad. It should also be pointed out that in Hiroshima the neutron kerma amounted to one fifth of the total kerma, and therefore the fraction of the absorbed dose due to neutrons would have been substantially lower than that due to gamma rays. In Nagasaki, the contribution of neutrons to the absorbed dose was much lower still. These facts suggest that the efficiency of neutrons for induction of microcephaly must be high.

146. Mental retardation, which is a consequence of substantial brain cell depletion at high doses, was similarly examined as a function of dose by Blot and Miller (24) in a fixed sample cohort of 1613 children exposed within 2 km of the hypocentre in the two cities. When they reached 19 years of age, they were tested for mental deficiency. The controls were children not exposed or exposed at distances of 3-5 km. Gestational ages between 6 and 15 weeks appeared to be most sensitive. Expressing the increase of mental retardation incidence as the ratio of the incidence observed in the sample to that observed in the two control proportions, the increase in Hiroshima in the total kerma range 200-299 rad was more than 100, while in Nagasaki it was only 12 in the same total kerma range and 53 for values of kerma higher than 300 rad. The difference between the two cities may have been due to the neutron component of the irradiation, which was almost absent in Nagasaki, or perhaps to other environmental differences between the two cities at the time of the explosions.

6. Malformations of the skeleton

147. In contrast with the paucity of quantitative data for many types of malformations, exhaustive descriptions of radiation-induced malformations of the skeleton of the mouse at several dose levels have been reported by Russell (296, 297) and Jacobsen (136). A less complete set of data on these types of malformations was reported by Novel (229).

148. The skeletal system is particularly suited for teratological experiments since bone embryonic primordia are sensitive to radiation and the defects are relatively easy to diagnose and quantify by staining procedures (136). Russell (296) has also pointed out that data on skeletal abnormalities could be used as an indicator of malformations of other important systems, such as the central nervous system.

149. It should be realized that the phenotypical variability of the skeletal system gives rise to quantitatively defined defects, even in normal unirradiated animals. This variability depends on a number of genetic and exogenous factors, to which radiation is added as a supplementary teratogenic agent. Malformations of the skeleton are usually classified according to *(a)* the anatomical region affection (skull, vertebral column, thorax etc.), *(b)* the topographic location (cervical, thoracic etc.), *(c)* the numbers of ossification centres and *(d)* their form (fusion, splitting, constrictions etc.). Complex systems of classification can be derived from the interplay of all these variables and exhaustive lists of possible defects are given in the papers cited above.

150. From the work of Russell (296) it appears that the developmental period of the mouse where skeletal abnormalities are induced extends from 6.5 to 13.5 days p.c. Each abnormality has its own period of maximum sensitivity. At the lowest effective dose, the period for each type of lesion lasts for only 1 or 2 days; for most malformations increasing the dose usually results in longer induction periods. Often biphasic or unusually long periods of maximum sensitivity are observed, probably indicating that developing organ primordia have a multitude of targets and that the same type of malformation may occasionally be produced by different pathogenetic mechanisms.

151. The results of Russell in 1956 (296) covered the exposure interval of 200 to 400 R and the whole of the period of major organogenesis in the mouse. From these results it was quite clear that the shape of the dose-response curves obtained at the period of peak sensitivity was different for each malformation.

152. Subsequent work (297) with BALB/C embryos irradiated on day 7.5 and on day 9.5 p.c., extended the exposure range downwards (25-100 R). At these exposures it was possible to differentiate better between those malformations which had a very high incidence at high doses and to search for the presence of thresholds. Concerning the day-7.5 series, the incidence of all malformations (of any degree) increased as a function of exposure, as shown in figure XXII. The author pointed out the difficulties in the interpretation of these curves due to the heterogeneity and the different degree of expression of the malformations. Even at 25 R, there was an increment in the frequency of abnormalities normally found, and furthermore, types of abnormalities normally not present in the controls were also observed. The significance of the incidence increment was assessed on the basis of two extreme and opposite assumptions: *(a)* that the malformations were completely uncorrelated with each other (i.e., by comparing the number of malformations obtained *versus* those expected in the

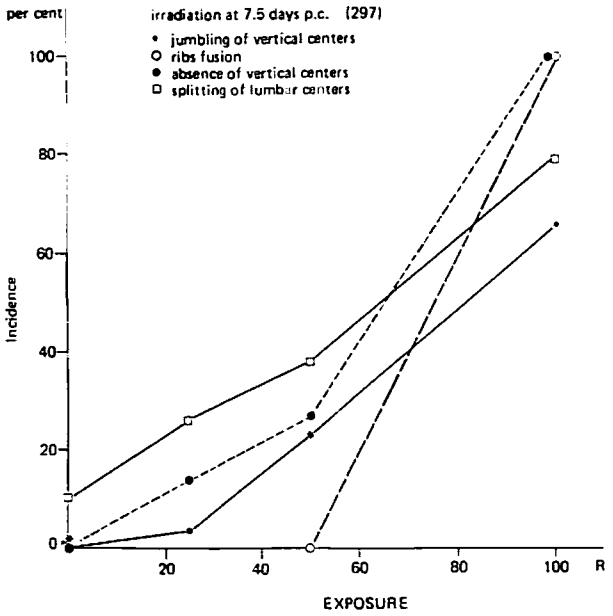

Figure XXII. Dose-effect relationships for incidence of skeletal malformations in the mouse

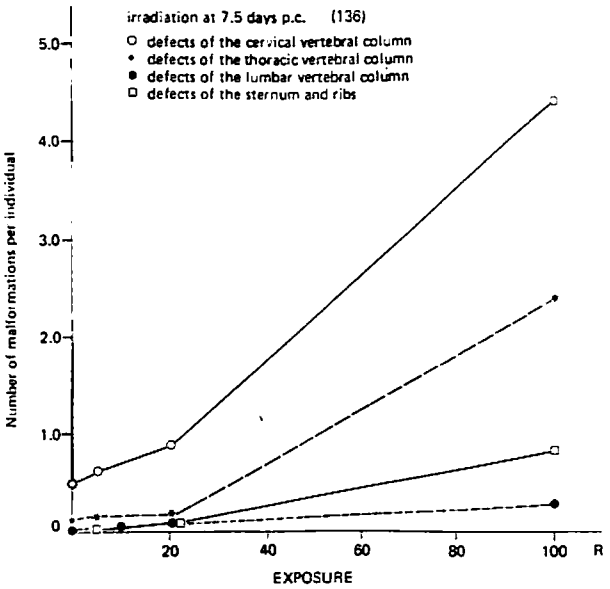

Figure XXIII. Dose-effect relationships for incidence of skeletal malformations in the mouse

control and in the 25 R group) and *(b)* that the malformations were completely correlated (i.e., by comparing the number of mice with one or more malformations in the two groups). The increment was shown to be significant under both assumptions, in spite of the small sample size, comprising 39 control and 30 irradiated animals. Similar considerations applied to four quantitative skeletal characters with irradiation at 8.5 days p.c.

153. Malformations of the vertebral column, with special regard to induction time, were studied by Murakami and Kameyama (205) on mice of the strains CF1 and ddN given a single 200-R exposure of x rays at 8-13 days of pregnancy. These authors reported a head-to-tail shift in the occurrence of malformations with advanced pregnancy stage (see also (208)) and the occurrence of two peaks of sensitivity in the vertebral abnormalities. The first peak was attributed to a disturbance of the early somitization, the second to a direct effect on the vertebral primordium itself. Interstrain differences in malformation frequency were noted, probably related to the rate of differentiation of the relevant structures. Other differences regarded the incidence of supernumerary or ectopic ribs, which were attributed to the genetic constitution.

154. The problem of the dose-effect relationship for skeletal malformations was the object of a thorough study by Jacobsen (136), who extended the range down to 5 R, thus covering the interval 5-100 R. After a detailed analysis of the abnormalities observed in different bones, where 5 and 20 R produced several defects, Jacobsen plotted, as a function of dose, the combined number of all malformations found in a given skeletal region (fig. XXIII). In all regions, an increase of exposure was associated with a rise of incidence. The lowest exposure tested, 5 R, produced an increase which was statistically significant at the 5-per-cent confidence level of the malformation incidence observed in all skeletal regions, when such increase was expressed by comparison with the appropriate controls. The experiment therefore did not establish the existence of a threshold, which might imply that exposures of less than 5 R could conceivably entail a risk of teratogenic damage (see also paragraphs 293 and 294).

155. Irradiation of the rabbit embryo (250 R on day 8-10 p.c.) has been reported to induce damage to the axial skeletal system (69). Dose-effect data for skeletal abnormalities in lambs were reported by McFee *et al.* (188). The experiments were carried out in the exposure range 100-400 R at day 23 p.c. The same paper includes a few data on cattle exposed on day 32 p.c. to 100-300 R, and also on the pig, with an average dose of 367 rad of neutrons received at different times (15-27 days p.c.).

156. Concerning information on humans, a roentgenographic survey of a small sample of 74 children exposed *in utero* during the Nagasaki explosion was performed in 1951 and 1952 by Sutow and West (339). These children had been exposed within 2 km of the hypocentre; the skeletal findings were compared with those of 91 children exposed within a distance of 4-5 km. There was no significant difference between the two groups. Furthermore, the occurrence of those skeletal anomalies which are found in animal experiments could not be documented in this human group.

7. Malformations of the extremities

157. The peak of sensitivity for the induction of malformations of the extremities by exposures in the interval 150-300 R, is found at 12 days p.c. in the mouse; the peak spreads over more days at higher exposures (fig. XXIV) (210). Sensitivity for digital malformations at 200 R is a maximum at 12 days of pregnancy (227), the morning after copulation being considered as day 1 of pregnancy. Considering the same morning as day 0, a careful series of experiments (343) has established the sensitivity peak to be at about 273 hours after mating, that is early on day 11

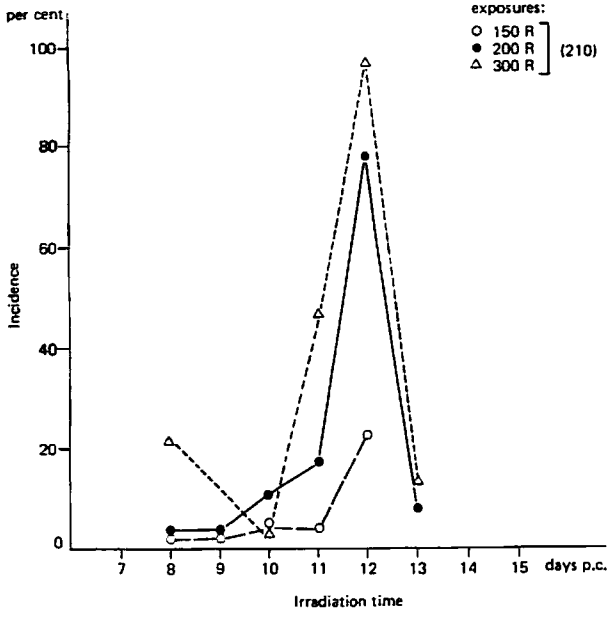

Figure XXIV. Incidence of malformations of the extremities in the mouse after irridiation at various times p.c.

(fig. XXV). The critical period for inducing abnormal development of the forelimb appears earlier and is longer than the corresponding period for the hind limb (294).

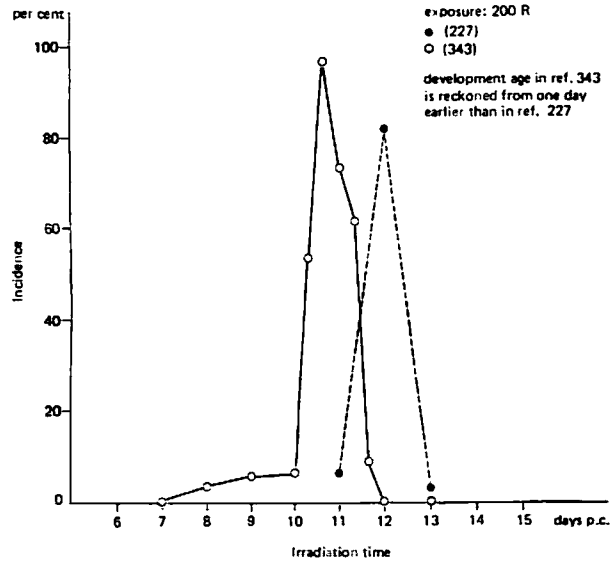

Figure XXV. Incidence of digital malformations in the mouse after irradiation at various times p.c.

158. Erickson and Murphree (82) described abnormal limb development in cattle, sheep and swine exposed *in utero* at various gestational ages to 200 R of ^{60}Co gamma rays. The period of maximum sensitivity was approximately day 32 p.c. for the cattle and day 23 p.c. for the sheep, in good correlation with the rudder stage of limb development. As in other species, the critical period for forelimb malformations occurred 0.5-1 day earlier than that for pelvic limb malformations.

159. Tail malformations are induced in the mouse by irradiation at a time between day 9 and 13 p.c. Following an exposure of 150 R, the peak sensitivity occurs on day 9 p.c. (210). When the exposure is increased to 200 R, the peak is on day 9-11 (210, 72), and with 300 R it extends to day 13 (210) (fig. XXVI).

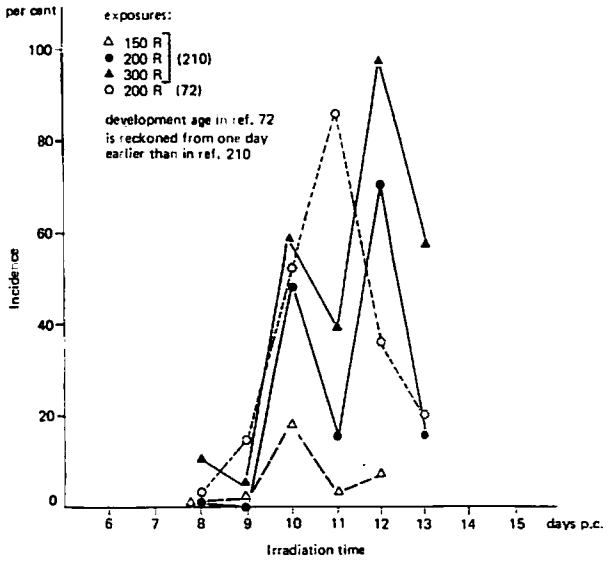

Figure XXVI. Incidence of tail abnormalities in the mouse after irradiation at various times p.c.

With larger exposures, therefore, the sensitivity peak becomes wider. Tail length is a parameter of interest because it can be associated with the number of vertebrae and may be scored by external inspection. Russell (294) reported results of experiments with exposures of 200-400 R at 4.5-13.5 days p.c., showing that the relative tail length is altered by irradiation on day 9-13 of pregnancy. Abnormal tail shape was in general correlated with tail-length effects. Jacobsen (136) found that, exposing on day 7.5 p.c. to levels in the range 5-100 R, the modal distribution of tail length shifted towards lower values with increasing exposure.

160. Warkany and Schraffenberger (357) described various types of skeletal abnormalities in the rat, among which malformations of the extremities were dominant. Depending on the radiation dose, these abnormalities were induced from 9-16 days p.c. On the whole, malformations of the long bones of the extremities occurred with irradiation at earlier stages, while toe and finger malformations were more common for later exposures. Similar observations were also reported by Wilson (362). Fused digits in forelimbs and hind limbs were observed in hamsters with an exposure to 200 R at 9.5 and 10.5 days p.c. (111) and in rabbits following 400 R on day 12-14 p.c. (59). Digital malformations appeared also in dogs following 150-200 R in the period 25-28 days p.c. (245).

161. Malformations of the tail, the extremities and various other abnormalities were examined as a function of exposure (150-300 R, 170-kV x rays) by Murakami *et al.* (210), irradiating mice at 8-13 days p.c. Dose-effect relationships of the threshold type were obtained for tail malformations; the highest yield was induced on day 12 p.c. With irradiation at this time, the induction increase in the range 150-200 R was about 1.3 10^{-2} R^{-1} (fig. XXVII), similar to that observed by Dagg (68).

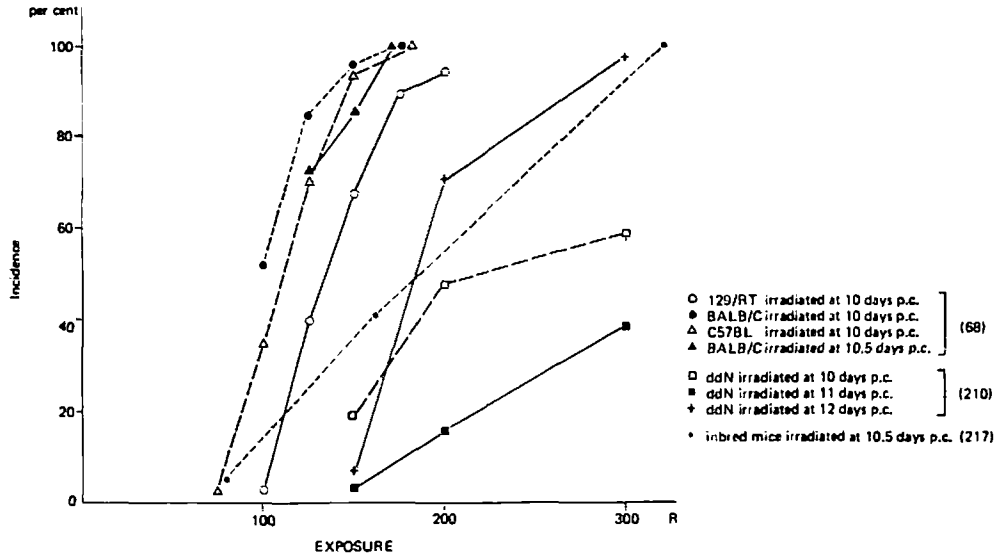

Figure XXVII. Dose-effect relationships for induction of digital malformations in the mouse

162. Malformations of the extremities were seen mainly in mice irradiated 11 and 12 days p.c., particularly following exposures of 200 or 300 R. A very detailed study of digital malformations in mice of the ddN and CF1 strains was performed by Nogami (227). Variables examined were the pregnancy time (8-13 days p.c. at a fixed exposure of 200 R), the exposure (150-300 R at a fixed time of 12 days p.c.) and the genotype. The incidence of individuals with malformations was proportional to exposure in the interval 150-300 R, at the rate of about $5\ 10^{-3}\ R^{-1}$ but below 150 R no data were obtained, which did not allow the assessment of a possible threshold (fig. XXVIII). Concerning the

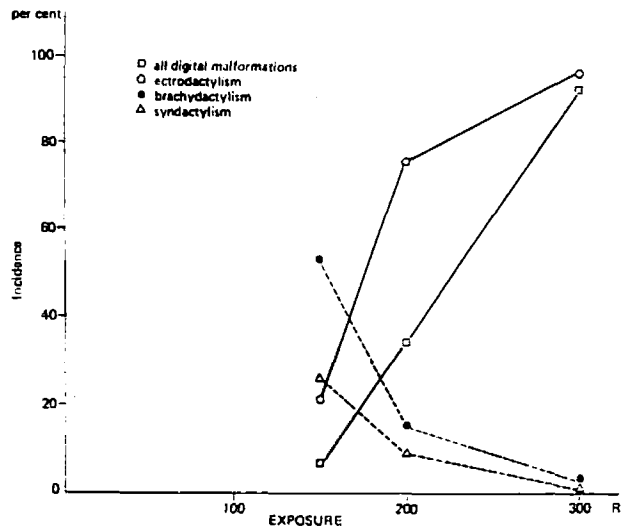

Figure XXVIII. Dose-effect relationships for various forms of digital malformations in the mouse (227)

The incidence of all digital malformations (□) was calculated over total living foetuses whereas the incidence of ectrodactylism (o), brachydactylism (•) and syndactylism (Δ) were calculated over foetuses with digital malformations only.

different types of malformations, ectrodactylism incidence clearly increased with dose following a threshold-type relationship, while brachy- and syn-dactylism displayed a decreasing frequency with dose (227).

163. Dagg (68) studied the induction of tail malformations and hyperphalangia of hind feet in three inbred strains and some reciprocal crosses. The defects were scored at 18 days p.c. on live foetuses that had been irradiated at 10, 10.5 or 11 days p.c. with exposures of 250-kV x rays in the range 75-200 R. Disregarding some differences between strains due to the variability in developmental age, the exposure-response curves for tail malformation were in general of the threshold type; the response increased linearly with exposures from about 75 to about 150 R (fig. XXVII). In this range the induction frequency can be calculated to be approximately $1.2\ 10^{-2}\ R^{-1}$, a value roughly applicable to the three strains used. The dose-effect relationship for malformations of the hind foot was more complicated. However, if the percentage of live litters with one or more hyperphalangous feet is plotted against exposure, the plot is consistent with a non-threshold linear relationship with a slope of about $5\ 10^{-3}\ R^{-1}$. Other plotting criteria could suggest curvilinear relationships. Mechanisms to explain the linear relationship have been suggested (68).

164. Nash (217) also observed a threshold-type dose-effect relationship for tail malformations in inbred mice following irradiation at 10.5 days p.c. with exposures in the range 80-320 R (fig. XXVII). Differences with respect to hybrid mice were also noted. No abnormalities of the feet were observed by external examination at exposures below 80 R, but at higher exposures overgrowth or reduction of the feet were clearly manifest. A different result was found by Jacobsen (136), who analyzed the degree of ossification of the paws, using alizarin-stained skeletons, and found effects at exposures as low as 5 R. He believed that the number of stainable centres of ossification in the extremities might be a suitable indicator, in sufficiently large samples, for studying the effect of small radiation doses. Limb malformations were reported to occur in marmosets given 250 R of x irradiation at 25 days of pregnancy, during the period of major organogenesis (249).

8. Other malformations

165. Malformations of the heart and great vessels of the rat were the subject of some early reports (295, 362). More recently, they were studied by Okamoto *et al.*

(233). The incidence of these malformations reached 85 per cent after a dose of 130 rad of 14.1-MeV neutrons, on day 8 p.c. Histopathological, cellular and kinetic changes of the relevant structures were described in other publications (199). Cardiac malformations were induced in dogs by exposures to 100-150 R at 21-22 days p.c. (245).

166. Malformations of the anus (small or imperforate) and of the urogenital system (hydronephrosis, convoluted or distorted ureter) were observed by Russell (294) in a small percentage of mice exposed to 200-400 R at 9.5 and 10.5 days p.c. In rats, Wilson (362) reported the occurrence of kidney agenesis after exposure to 100 or 200 R on day 9 p.c., and horseshoe kidney after irradiating on day 10 p.c. Both types of malformations were induced also on day 11 p.c. A 77-per-cent incidence followed an exposure of 200 R. Urogenital malformations (unilateral or bilateral kidney agenesis) with a peak sensitivity just before the appearance of the metanephric kidneys were reported in the dog (245).

167. Thoracic and ventral hernia in mice were described by several authors. Murakami *et al.* (209) obtained an incidence of about 5 per cent with 200 R on day 9 p.c. In a subsequent experiment (210) a maximum incidence of 44 per cent was obtained with 300 R on day 8 p.c. Jacobsen (136) described an incidence of about 2 per cent following an exposure of 100 R at 7.5 days p.c. In hamsters, Harvey and Chang (111) reported a 12-per-cent incidence of hernia and spina bifida following 200 R on day 7.5 p.c.

9. All malformations

168. In spite of the great interest for risk assessments of the overall induction of malformations, very few authors have given figures for the total percentage of malformed conceptuses in their experiments. Taking all malformations together, the peak sensitivity to induction occurs on day 9 p.c. in the rat embryo (325). In the dog the time immediately following implantation (corresponding to the primitive streak stage and early organogenesis) appears to be the most sensitive for the production of malformations (245). Concerning dose-effect relationships (fig. XXIX), Murakami and

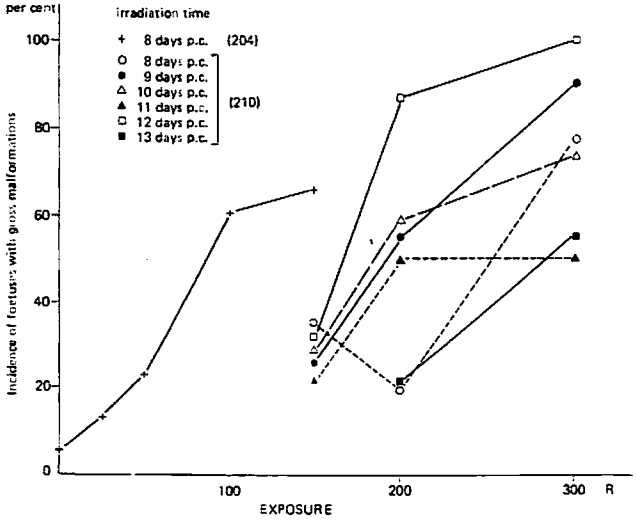

Figure XXIX. Dose-effect relationships for induction of gross malformations in the mouse

Kameyama (204) reported the incidence of grossly abnormal mouse embryos (living and dead on day 13 p.c.) following irradiation with various exposures (25-150 R) on day 8 of pregnancy. From this study the induction can be calculated to be about $5\ 10^{-3}\ R^{-1}$ without any apparent threshold. In a later report (210), Murakami gave the incidence of abnormal foetuses reaching 19 days p.c. for an exposure range of 150-300 R, with irradiation occurring in the period 8-13 days p.c. The induction in this range (discarding dead foetuses) appears to vary between $1.9\ 10^{-3}\ R^{-1}$ and $4.5\ 10^{-3}\ R^{-1}$, depending on age at irradiation.

169. Recent human studies relevant to all types of malformations deserve attention. Kinlen and Acheson (148) published a retrospective study of the irradiation history of 379 women hospitalized for spontaneous abortions and of 605 women who delivered malformed children. Their radiation history was compared with that of a control group. No significant difference was found. The sample size was clearly too small to determine the effects of doses at the diagnostic level. The authors concluded that, under the conditions of observation, there was no evidence that irradiation could play an important part in the etiology of abortions requiring hospitalization or in the induction of congenital malformations. Hagstrom *et al.* (103) made observations in about 700 women who received ^{59}Fe during pregnancy for metabolic studies, resulting in an estimated dose to the foetus of 5-15 rad and also in about 700 controls. Their findings were essentially negative for the induction of malformations in the offspring.

170. More recently, Diamond *et al.* (74) reported on a prospective study of about 20 000 white and black children who were exposed *in utero* over the period 1947-1959 in the course of abdominal irradiations of their mothers, mainly for pelvimetry, placentograms or simple radiography of the abdomen. The children were traced in 1961-1967 to determine their mortality and the cause of death according to the death certificates. The group was compared with a non-irradiated cohort of approximately 36 000 children matched by hospital and time of birth, sex, parity and race. A sample of about 152 000 person-years in the exposed group and 28 000 person-years in the control were thus collected and examined. The study was mainly concerned with leukaemogenesis, but data on malformations were also available and have been summarized by Lilienfeld (172). No statistically significant difference in the mortality from congenital malformations was seen between exposed and control children. Concerning the sensitivity of different gestation periods, there was no difference in the malformation frequency between two groups defined by the trimester at which they were irradiated. Within each trimester, the irradiated children had a slightly higher incidence of abnormalities than the controls, in both sexes and races. In white children, the mortality of the exposed group was almost twice that of controls (183 per 10^5 person-years against 98 per 10^5). In black children, on the contrary, the same mortality was observed in exposed and control groups (212 per 10^5 person-years and 229 per 10^5 person-years, respectively). The difference could not be attributed to congenital malformations.

171. In a prospective study (235), the morbidity and mortality of about 1000 white children exposed *in utero* (average foetal dose about 700 mrad) during routine diagnostic pelvimetry were followed for 15 years, compared to an adequate control group. The data (which had been reported previously by Griem *et al.* (99, 100)) were most carefully reanalysed and compared with previous studies, but were insufficient to associate radiation conclusively with any effect. It should be pointed out that only 3 children were exposed during the first 50 days of gestation and only 24 during the first trimester, while 87 per cent were irradiated during the second half of pregnancy. With this sample size, radiation effects could be detected only if they were induced in excess of 1 per cent, which is at least an order of magnitude higher than might be expected for the most frequently radiation-induced abnormality in man, namely microcephaly (see paragraph 144). In the opinion of the authors, the study is therefore of value in excluding the possibility that doses of the order of 1 rad or less could produce effects exceeding 10 times the incidence observed at high doses.

172. Oppenheim *et al.* (236) reported a comparison between studies in which the exposure to radiation was the result of medical indications (74) and studies involving non-selective exposure to radiation (100, 145, 235). Significant discrepancies were found, suggesting that the data reported in the first instance could be attributable to the bias introduced by the medical conditions requiring the exposure, rather than to the exposure itself. These new data therefore cast some doubt on the validity of the studies of diagnostic exposures and suggest that such exposures may actually be less harmful than previously assumed (236).

173. Neumeister (222) reported most recently on a series of 37 cases of embryonic exposure between week 1 and week 10 p.c. up to a maximum of 20 rad. These cases had been referred to a specialized unit of clinical radiobiology for expert opinions concerning a possible abortion. Of the 35 cases that were conducted to term, only 15 children were examined at 1-3 years of age. In all these cases the pregnancy had proceeded normally, the weight and body size and the subsequent development of the children were found to be normal and no difference was noted between the exposed individuals and their brothers or sisters. In one case where the exposure had been less than 1 R at 4-6 weeks p.c. a talipes calcaneus was observed, but a causal relationship with irradiation was excluded. A second case (exposure 3-5 R at 2 weeks) showed talipes calcaneus and a lumbar myelomeningocele; a relationship with the previous exposure was estimated to be improbable. In spite of the accuracy in the estimates of exposure time and of dose calculations, the size of this series is clearly too small to be of any general significance.

174. In connection with the possible effects of very low doses of radiation, some correlations have been attempted between the overall incidence of malformations in man and the level of natural radioactivity. In what they regarded as a feasibility study, Kratchman and Grahn (158) tabulated the incidence of deaths from congenital malformations for the years 1952-1956 in various areas of the United States of America and grouped these data by geologic province. They were able to show a higher mortality from malformations in the geologic provinces containing major uranium ore deposits, uraniferous waters or high helium concentrations. It should be pointed out that no measurements of radiation levels were made and also that only crude statistical indexes were used, without corrections for birth rate, maternal age, death rate or other factors that could influence the observed correlation. The data, however, were considered by the authors to be sufficiently provocative to justify further investigations.

175. In a highly speculative paper Wesley (361), took a different approach: he analysed the fraction of malformed births over the earth as a function of geomagnetic latitude, which is associated with different doses from cosmic rays. The relation between the world-wide incidence of congenital malformations obtained from vital statistics and the geomagnetic latitude appeared to be highly significant. The lines of equal incidence of malformations were said to match the corresponding lines of the magnetic field of the earth. According to the author, all congenital malformations could be attributed to "background" radiation (presumably its extraterrestrial component). It should, however, be pointed out that both the background radiation data and the epidemiological data on which much of this paper rests have been seriously questioned (46, 330).

D. OTHER EFFECTS

176. **Fertility and fecundity.** Male CF1 mice were exposed to 25 or 100 R in the period 0.5-18.5 days p.c. and tested for reproductive potential at ages ranging from 2 to 8 months. With the exception of a reduction of the reproductive quotient in animals exposed to 100 R at 10.5-12.5 days p.c., no significant effect on fertility was documented. In the opinion of the authors, the small reduction of the reproductive quotient could reflect a general disability of the animals, rather than a reduction of their true reproductive potential (288).

177. Concerning effects in the female, some rather incomplete data ae available for mice and rats. Female mice were exposed during the first and the second half of the organogenesis to 20-80 R per day for 5 consecutive days. Their reproductive capacity was then tested in a successive generation. Irradiation on day 11-15 p.c. was shown to have the greatest effect and the groups exposed to 60 and 80 R per day were unable to reproduce further. Animals exposed to 40 R per day produced litters with very high mortality, and the number of litters produced depended both on exposure and on gestational age at irradiation. The frequency of litters was particularly small among animals irradiated in the embryonic stage. The average litter size, on the contrary, was not greatly influenced by irradiation, the differences in the reproductive capacity being mainly reflected by litter frequency rather than by litter size (166). Rat females exposed *in utero* to 110 R on day 15 p.c., to 150 R on day 18 or 20 p.c., or to 220 R on day 17-20 p.c. were subsequently mated to normal males. These irradiations had no effect on the incidence of infertility and on the number of offspring produced.

The only effect consisted in a significant (20 per cent) reduction of the ovulation rate, which was dose-dependent (212).

178. Cataract formation has been reported as a result of exposing mice to about 100 R during the embryonic stages (270, 292). The induction of tumours following irradiation *in utero* is discussed in Annex I.

179. A significant decrease of the *in vivo* and *in vitro* incorporation of labelled aminoacids into proteins, 24 hours after irradiation, has been shown in embryo rats as a result of a dose of 180 rad on day 13 p.c. (313). Enzyme changes appearing post-natally in the brain and the liver of rats irradiated *in utero* (25 or 50 R on day 14 p.c.) have also been described (214, 215, 216). Concerning haemopoiesis, an arrest of cell division accompanied by alterations of the haemoglobin synthesis in the embryonic erythroid cells has been shown to occur in the mouse after a single x-ray dose of 120 rad received on day 10 p.c. (85).

180. Ultrastructural effects induced by 50 R in the liver cells of the rat embryo on day 13 p.c. and consisting essentially in changes of the aggregation of polysomes and in a delay in the appearance of the smooth endoplasmic reticulum provide some insight into the chain of molecular, biochemical and structural events taking place after pre-natal irradiation (62). Some information concerning the effects of radiation on the regenerative capacity of the muscle are also available. The disorders observed were more pronounced in animals irradiated during the second half of pregnancy, when histogenesis of the muscle occurs (163).

IV. THE FOETAL PERIOD

A. LETHAL EFFECTS

1. Mouse

181. The foetal period in the mouse begins approximately at 13 days p.c. It should be pointed out that many of the experiments on the induction of mortality during the major organogenesis period reviewed in paragraphs 84-108 covered some of the later stages as well. As the foetal development proceeds, the dose necessary to kill animals before or after birth increases gradually, particularly the pre-natal LD_{50}. The percentage of abnormal animals with a given dose, on the other hand, tends to decrease with foetal age.

182. Rugh and Wohlfromm (286) established that the pre-natal LD_{50} for irradiations at day 14-17 p.c. is higher than 700 rad (see table 4), with a clear tendency to increase with foetal age. Their experiments show that killing the foetus *in utero* requires very high doses, most of the lethality at lower doses being seen at birth or just after birth. Irradiation of the foetus results in fewer macroscopic anomalies than irradiation of the embryo, being instead consistently associated with stunting. These data are similar to the results of Dekaban (72), who exposed foetal mice to 200 R and scored the percentage of surviving and apparently normal animals. This indicator was shown to rise from 38 per cent for irradiation on day 13 p.c. to around 100 per cent for irradiations on days 17 and 18 p.c. The mean litter size, serverely affected by the exposures of the same magnitude during the embryonic period, gradually increased to normal for irradiations during the foetal stage. Experiments with fractionated (152, 155) or chronic irradiation (154) also show clearly that less lethality is induced during the foetal development than in earlier stages.

183. The study of post-natal lethality induced by irradiation in the foetal stage is a necessary complement to the experiments on *in utero* mortality. Rugh and Wohlfromm (289) extended their previously reported observations (286) to the first 30 days of life. They showed that a gradual increase of the post-natal $LD_{50/30}$ takes place with increasing post-conception age at irradiation from about 250 R on day 13 p.c. to about 600 R on day 18 p.c. The second day after birth showed the highest death rate. From these data it can be concluded that the late foetus is at least as radioresistant as the mature animal. Although limited to only 2 days in the foetal stage (14.5 and 17.5 days p.c.), the data of Nash (217) are also useful to compare the resulting pre- and post-natal viability with that observed with irradiation at earlier stages. They show a decline of sensitivity with increasing foetal age for induction of pre-natal and post-natal lethality, together with a slightly higher resistance of hybrid, as compared to inbred, genotypes. No differences of sex could be shown. Similarly, the data of Konermann *et al.* (155) on fractionated exposures at 40-80 R per day during the main developmental phases show that post-natal survival is only slightly affected. This observation is particularly striking when compared with the effect of the same exposures during organogenesis. Again, the first week after birth is the time when most deaths are observed.

184. Long-term survival was studied by Upton *et al.* (347), who reported that exposure to 300 R at 14.5 days p.c. produced much more life-span shortening in males than in females. Minimal reductions of longevity after exposure at later stages of gestation were reported. Nash and Gowen (219) also emphasized the effect of sex in the survival as adults of mice irradiated during the foetal stage.

185. Rugh and Wolff (293) claimed that an exposure of 10 R during the foetal stage increased the survival of mice receiving a mid-lethal dose at an age of 4 months. The age dependence of this beneficial effect is however uncertain, and higher exposures (25-300 R) to foetuses of any age are detrimental to survival after test doses. Christensen and Jackson (64) exposed mice 14.5 days p.c. to 150 R and subsequently determined their $LD_{50/30}$ at the age of 8 weeks. The data suggested that between 30 per cent and 53 per cent of the foetal exposure had been repaired by that time. The authors also concluded that the foetal stage was considerably more sensitive than the adult in terms of irreparable radiation damage, probably of the haemopoietic system. These results should be compared with the data of Sikov *et al.* (322) and others in the rat (see paragraphs 189 to 191).

2. Rat

186. In the rat the foetal period begins approximately 16 days after conception. The data in this species are far less complete and often limited to a few ages in the foetal period (see table 4). They are, however, self-consistent and in agreement with the results obtained in the mouse.

187. Murphree and Pace (212) irradiated with a few selected exposures (110, 150, 220 R) at ages from 13 to 20 days p.c. Their data show an increased resistance to post-natal lethality as a function of age. The majority of post-natal deaths were observed, as in mice, within the first week. Reincke *et al.* (253, 254) determined carefully the $LD_{50/30}$ of rats irradiated 5 days or 1 day before birth, confirming the increase of radioresistance of the foetus with age, and the absence of any sex effect. Sikov *et al.* (322) examined the effect of 50 or 185 R on day 15 p.c., showing that the incidence of still birth and neo-natal mortality was larger with the highest exposure, and that most live-born animals died within the first week. The post-natal mortality of the rat foetus was also studied by Ader and Deitchman (1), who used exposures of 200 R on day 16 p.c. It should be pointed out that in spite of similar values of $LD_{50/30}$ for the adults in both species, the $LD_{50/30}$ for the foetal rat is between 200 and 300 R and therefore consistently lower than that for the foetal mouse, which appears to reach 600 R during the late gestational ages.

188. In 1954, Russell (295) summarized the information on the mode of death of various animal species exposed during the foetal period. From the summary it may be concluded that the syndrome of haemopoietic failure is the most common cause of death at doses around the LD_{50}. More recent reports do not add further quantitative data on this subject.

189. Reincke *et al.* (254) studied long-term survival following foetal irradiation. They found that exposure to 220 R 5 days before birth resulted in 53 per cent survival after 1 month of life, and that the subsequent survival, up to about 4 months, was practically the same (48 per cent). The long-term survival of these animals was not significantly different from that of controls.

190. Sikov *et al.* (322) reported that the post-weaning mortality of female rats exposed to 185 R on day 15 p.c. was significantly higher than that of controls, while the median life span of males was not significantly affected, thus pointing again to a dependence of the long-term mortality on sex. An interesting observation in these experiments is that the $LD_{50/30}$ of an irradiation at 100 days of age decreases as a linear function of the dose of a pre-natal irradiation at all developmental ages. In the opinion of the authors, such a linear dependence would imply a general decrement of fitness as a result of pre-natal exposure.

191. According to Maisin *et al.* (180) the radiosensitivity of rats surviving an exposure of 70 R at 10 days p.c. is lower than that of non-irradiated control animals. Although a tentative interpretation was advanced, based on the larger elimination of the more sensitive animals by the first dose, no definite conclusion was drawn.

3. Human experience

192. Some studies have been made on the termination of pregnancy in women irradiated in the pelvic region because of various pathological conditions or for therapeutic abortion. These studies provide qualitative information on the mortality of irradiated human embryos or foetuses. The early observations of Murphy (213) are not very relevant for this purpose, but the data of Harriss (106) on therapeutic abortion are somewhat more significant. These data covered 138 cases of women irradiated at 6-18 weeks of pregnancy with exposures of 510 R of 200-kV x rays in the uterine fundus. The treatment caused interruption of pregnancy in 129 cases, within an average time of 4 weeks. The foetuses were grossly macerated and in one case a cyst of the brain was found. Non-specific degenerative changes in the placenta were also noted. Mayer *et al.* (187) reported that a single exposure of 360 R in the uterus is sufficient to cause abortion in the large majority of cases.

193. Autopsies of irradiated human foetuses are rare. Driscoll *et al.* (76) reported two cases where the mothers had been treated with radium for cancer of the cervix. The foetuses, measuring 15 and 21 cm, had been exposed to about 800 and 1600 R, respectively. One was examined 2 days and the other 10 days after the beginning of the irradiation. Damages to the nervous and the haemopoietic systems were observed, and the alterations of the mesenchymal cells extended to regions of the body where the estimated exposures had been of the order of 50 to 100 R. This pattern of damage is not unlike that described for other species.

194. The data on mortality of children irradiated *in utero* as a result of the Hiroshima and Nagasaki explosions are of epidemiological nature and are possibly more informative. Yamazaki *et al.* (371, 372) reported on the outcome of pregnancy of 98 women exposed in Nagasaki. Among 30 women with major signs of the radiation syndrome who were within 2 km of the hypocentre, 7 ($\sim$ 23 per cent) foetal deaths and 6 (20 per cent) neo-natal and infant deaths were observed. Of the 17 surviving children, 4 were mentally retarded. The overall morbidity and mortality was therefore about 60 per cent in this group. In another 68 mothers exposed within the same distance but with no signs of radiation syndrome, the overall mortality was 10 per cent, only slightly above the mortality of the control group (6 per cent). In the second and third trimester of gestation, the foetal, neo-natal and infant mortalities were significantly elevated among the offspring of mothers who suffered from the radiation sickness. The evaluation of these data is, however, very difficult, owing partly to the absence of proper dosimetry and partly to the possible effect of the blast and thermal radiation, as well as to the indirect effects of maternal haemorrhagia and infection.

195. Kato (145) studied the mortality at later stages of life, following for 24 years 1300 children exposed *in utero* in Hiroshima and Nagasaki. He showed a dose-related increase of mortality during the first year of life, an absence of any relationship between mortality and dose from 1 to 10 years, and again a dose-related

increase at later ages. These increases of mortality with dose were not attributable to concomitant variables, such as age of parents, birth order, socio-economic factors etc. Mortality after 10 years was not associated with any specific cause of death and, contrary to what might have been expected from the animal data, radiation mortality was only apparent among children exposed in the third trimester, possibly because of a higher rate of abortions and still births of embryos exposed earlier. Dosimetric uncertainties concerning exposure *in utero* do not allow more definite conclusions.

B. DISTURBANCES OF GROWTH

1. Mouse

196. Generalized and local growth disturbances are probably the most commonly described effects of irradiation during the foetal stages. The following review is rather arbitrarily limited to those reports paying special attention to growth defects.

197. Duplan and Izadian (78) studied pre-natally the body and liver weights of foetal mice exposed between day 14 and 18 p.c. to 200, 500 or 800 R and sacrificed 1-4 days after irradiation. The body weight was lowest for the higher exposures and for irradiation at early age p.c., the effect being more marked with long observation times. The liver weight, on the contrary, was most depressed when the interval between irradiation and sacrifice was short, suggesting that the effect was temporary.

198. In the experiments of Rugh, Duhamel *et al.* (271), mouse stunting was studied systematically with exposures of 100 R at various times from fecundation to 18 days p.c. The average weight of the animals at an age of 4 months was minimum for irradiations at 12 or 13 days p.c. (females and males, respectively). Although mature weight tended to increase with increasing foetal age at irradiation, it never reached control values in either sex. When followed to 35 months of age, the average weight of the animals was still depressed compared to controls. The dependence of this depression on gestational age at irradiation was very similar to the weight depression observed at 4 months of age (272). A "linear" decrease of body weight one day after birth with dose in the range 120-240 rad for irradiations on day 15 p.c. was reported by Hazzard and Budd (117). The body weight of the rats, averaging 6-8 g in the control new-borns, decreased to about 3-6 g with 240 rad. However, if the control weight is taken into consideration, the trend of the curve is clearly curvilinear.

199. With respect to fractionated irradiation, Konermann *et al.* (155) reported that the average post-natal weight of mice exposed to 40, 60 or 80 R per day during the foetal period was always below control in the 3 weeks after birth, with a maximum depression in the first week. The effect of the exposure rate appears to be minor. Post-natal growth measurements referring specifically to the skeleton are also available in the mouse, where stunting of several bones may be traced until sexual maturity (271).

2. Other animals

200. Sikov *et al.* (322) reported a small decrease in the birth weight of rats after exposure to 50 or 185 R on day 15 p.c. This difference persisted to an age of 80 to 100 weeks, both in male and female animals, and was more pronounced with the higher exposure. The weights of the brain, spleen, thymus and kidney were also depressed in the irradiated animals. Martin (186, 183, 184, 185) carried out a series of experiments on the response of the whole-body and organ growth in animals exposed at 18 days of gestation to 160 or 220 R of gamma radiation. Depression of the whole-body weight relative to controls was maximum at 21 days, but persisted up to 1 year of age, a time at which the weight was about 70 per cent of that of the controls. Although most organs were lighter in irradiated animals, those which were consistently different from controls (when referred to the body weight) were the brain, testes, pituitary and adrenals. The number of cells in the various organs, between 7 and 60 days, expressed as per cent of control, indicated that the kidney and liver followed in the main the body weight changes. Spleen and thymus, on the contrary, followed different patterns, reflecting presumably the sensitivity of the specific cell populations and their recovery kinetics. Other results of experiments on body weight of rats can be found in Ader and Deitchman (1). They used exposures of 200 R at 16 days p.c. and followed the body weight during 60 days of age.

201. Rugh, Duhamel *et al.* (273) followed the body weight during 23 months of life of two monkeys which had been exposed in the foetal stage to 200 and 300 R. The organ weight at the autopsy and some skeletal measurements were also reported. All the measured indicators failed to attain the normal values.

3. Human data

202. Only the general effects on human post-natal growth are reviewed in this section, the special case of microcephaly having been already considered in paragraphs 141-146. The data available regard the exposures of A-bomb survivors in Japan and, to a lesser degree, medical exposures.

203. About 1700 Japanese children exposed *in utero* to radiation from the atomic explosions were followed on an annual basis (366, 367, 368). More than 80 per cent of these children were subjected to anthropometric measurements and various other tests when they reached 17 years of age. They were subdivided into three major comparison groups: those exposed between 0 and 2 km from the hypocentre, those exposed between 2 and 5 km from it, and unirradiated controls. The group nearest to the hypocentre was further classified according to the estimated dose to the mothers (more than 25 rad and less than 25 rad (18)), and according to the presence of acute irradiation symptoms. In those exposed within 1.5 km, a reduction of mean head circumference (1 cm, or 2 per cent) was observed; additionally, the height and body weight, in both sexes and in both cities, were also reduced by 2.3 cm and 3 kg, respectively. In most cases the incidence of the effects was significant. Other indicators, such as the chest

circumference and the intercristic diameter, were also affected, although more irregularly. The analysis of induction by trimester of gestation revealed no consistent pattern.

204. In another study, 286 adolescents who had been exposed *in utero* at Nagasaki, were examined for growth and developmental defects (48). The indicators were the age at menarche, the degree of epiphyseal closure in the wrist and several other body measurements, such as head circumference, standing and sitting height, and weight and chest circumference. Numerous differences were found that were consistent with a postulated radiation effect. With regard to pregnancy stages at irradiation, no significant differences were observed in the males, but almost all measurements in the females exposed during the first trimester were significantly greater than in those exposed during the last trimesters. Head size was the single anthropometric indicator most significantly affected.

205. Another study, by Russell *et al.* (306), specifically concerns bone maturation. Here, 556 subjects exposed to the A-bomb explosions over Hiroshima and Nagasaki were examined to record the age at which epiphyseal closure of the wrist bones occurred. In both boys and girls, closure was found to take place 6-9 months later that in other Japanese children and in American children. Possible contributory factors were discussed. However, contrary to previous reports (48), no correlation was seen between the dose to the mother and the time of closure of the children exposed *in utero.*

206. Shohoji and Pasternak (312) used an elaborate Gompertz function to characterize and analyse the growth of about 900 children exposed *in utero* in Japan and to test for differences between sexes, cities, trimester of exposure and exposure groups. Differences observed between groups exposed at various post-conception times were found almost without exception to be non-significant. Differences between exposure groups were greater in males than in females, a fact pointed out earlier (368), but there was no consistent relationship between growth impairment and exposure group. These new findings provide some support for the previously reported retardation in stature observed in pre-natally exposed subjects (48, 368), but the authors believe that this support is inconclusive.

207. A comprehensive evaluation of all these studies indicates beyond doubt that reductions of body size and growth can be induced by radiation in humans. Moriyama *et al.* (200), Belsky *et al.* (20) and Blot (23) have identified the following effects on growth as characteristic of *in utero* exposure of children: *(a)* reduced height and reduced head and chest circumference, effects which are especially pronounced at high doses and persist to maturity; *(b)* microcephaly and mental retardation, which are more frequent in children exposed within the first trimester and for which the dose-effect relationships were discussed in paragraphs 141-146.

208. Regarding medical exposures, Nokkentved (228) reported on 152 Danish children who had been irradiated within the first 4 months p.c. in the course of diagnostic procedures, compared with 143 non-exposed siblings. Several types of x-ray procedures were involved, only a few being simple pelvimetries. In general, the irradiations were delivered in the course of only one examination, but in about 40 cases multiple examinations were performed, up to five in 2 cases. It was found that 15 of the irradiated children (9.9 per cent) and 13 of the controls (9.2 per cent) presented a variety of malformations, including heart malformations, spina bifida, inguinal hernia, microcephaly etc. Both the radiation dose estimated by the number of radiographs and the time at irradiation could not be clearly correlated with the incidence and type of malformations. However, the height of the children irradiated during the second month of gestation was clearly smaller than control, and this finding could not be explained by a number of biological, medical or social factors taken into consideration. Furthermore, the weight at birth of children irradiated during the second month appeared to be low, although the information was incomplete because this indicator was not determined in all children. This study confirms therefore the existence of a growth-retarding effect of irradiation during the second month of pregnancy. However, it does not contribute to improve the information on the incidence of malformations, because of the relatively small sample size.

C. MALFORMATIONS

1. General

209. It is widely accepted that following the termination of major organogenesis, the effects of irradiation become gradually more subtle and more difficult to document, at least macroscopically. The large number of reports on malformations induced during the embryonic stages (see chapter III, section C), compared with the few papers on teratogenesis during foetal development, is in itself a demonstration of that fact.

210. However, that statement, like any general assertion, needs to be qualified. In fact, the distinction usually drawn at the whole-body level between embryogenesis (implying differentiation of new structures) and foetal development (mainly involving the growth through cellular division of the newly differentiated structures) is hardly tenable at the level of single systems or organs. At any given time, new structures can still be formed while others are simply expanding; some organs may still be undergoing differentiative processes while others are enlarging. In addition, the period of maximum sensitivity to induce malformations of certain embryological structures, which at low doses is characteristically confined to the time of maximum differentiation, can be longer with sufficiently high doses (see paragraphs 28-29). Therefore, some malformations are in fact induced with irradiation at ages conventionally regarded as part of the foetal stage.

211. In general, the malformations appearing with increasing foetal age are more subtle and resulting functional defects gradually less pronounced. That influences the fate of the malformed offspring, since major abnormalities produced during organogenesis may be lethal during the intra- or the early extra-uterine life,

while less serious defects, produced at the foetal stages, may be compatible with life, although more or less seriously disabling.

2. Animal data

212. The considerations made in paragraphs 121-123 naturally apply to malformation induction during foetogenesis, but the extreme paucity of data does not allow any analysis of the time and dose dependence of the effects. Very few of the reports examined in chapter III, section C, cover also the induction of teratogenic effects in the foetal period.

213. Concerning **external head abnormalities**, Dekaban (72) reported that an exposure of 200 R in the mouse induced small or deformed heads in 14 per cent or 4 per cent of cases, if irradiation took place at 14 or 15 days p.c., respectively. No effect was seen for irradiation at later ages. Few observations have been reported on the induction of **ocular defects** in the foetal stage. Hicks *et al.* (122) carried out experiments with a few rats irradiated on days 16-20 p.c., showing a reduction of the ocular diameters and microscopical retinal changes (rosette formation).

214. Relatively more abundant are the data on malformations of the **central nervous system**. In the mouse, the induction of gross abnormalities by irradiation with 200 R after day 13 p.c. is less common than in previous stages but still present (72, 143). Microscopically, however, malformations can be ascertained far more accurately, and most of the brains prove to be abnormal with heterotopias, deformities and architectural alterations of various kinds, for irradiation at times at least up to 17 days p.c., although with progressively decreasing incidence (72, 73). The papers by Hicks *et al.* (121, 122) provide a good morphological description of the cerebral lesions in rats following an exposure of 200 R during the foetal stages.

215. These data, together with those of Satow and Miyabara (308), Kameyama and Hoshino (143), Hoshino *et al.* (126), Hayashi *et al.* (116), Mullenix and Norton (202), are of great interest for establishing the pathogenesis of the malformations or their relation to behavioural disturbances, but are of limited value for the purpose of establishing dose-effect relationships. There is, however, unanimity on the significant fact that the developing structures of the central nervous system remain highly sensitive to teratogenic damage until late in gestational life, when gross malformations of other organs may no longer be induced.

216. There are very few data for the induction of other types of malformations by irradiation of other mammals during the foetal stage. In conclusion, it appears that some gross malformations can be produced by irradiation during the foetal stages, particularly in the earliest phases and with doses of a few hundred rad. With increasing age, however, the foetus becomes progressively more resistant to their induction. Microscopic analysis of the anatomical structures, particularly the brain, shows the presence of lesions or architectural defects which would otherwise be undetectable by external examination of the embryos. The incidence of these deformities also decreases as the maturation of the foetus proceeds to completion.

3. Human data

217. In man, the malformation most consistently reported for irradiation during the foetal stages is microcephaly. Its production however, is not specific to the foetal period; on the contrary, the experience in animals shows that the time of maximum sensitivity occurs during embryogenesis. For that reason, both the animal and the human data relevant to this malformation were reviewed in paragraphs 141-146. It is however appropriate to point out that, particularly with regard to man, most of the information reviewed in those paragraphs applies to foetal irradiation as well.

218. A special case of malformation reported in the human after irradiation during the foetal period is segmental heterochromia of the iris. Lejeune *et al.* (169) preliminarily described their findings in a group of about 2800 children, including controls and children of mothers who during pregnancy had been irradiated for diagnostic reasons in the abdomen or the pelvis. Considering the whole population observed, 8 out of 567 irradiated children and 2 out of 2276 controls had heterochromia of the iris. When the comparison was limited to two smaller groups, 3 out of 421 irradiated and 1 out of 448 control children were found with this condition. It appeared that the critical period of the induction of heterochromia might be the 4th and 5th months of gestation.

219. Cheesman and Walby (63) retrospectively studied 7813 children, 4-7 years old, who entered school in Belfast in 1961. Of these, 67 (0.86 per cent) had heterochromia of some sectors of the iris. No significant difference in incidence was found between children who were reported by their mothers to have been irradiated for diagnostic purposes *in utero* (1.17 per cent) and the non-irradiated controls (0.81 per cent). Among irradiated boys the prevalence of heterochromia was slightly, though not significantly, higher than among the non-irradiated boys. That situation was not observed in girls. Eight affected boys had been irradiated *in utero*, and in 7 of these the irradiation had been performed during the 7th month of gestation.

220. Jacobsen and Mellemgaard (138) studied the offspring of 201 female patients irradiated in the gonadal region in 1924-1930 for radiological urinary examinations or for assessment of the position of the foetus. The offspring of these patients were subdivided into 250 born prior to irradiation of their mothers, 46 exposed *in utero* and 169 conceived after irradiation. No significant differences were found in the number of abortions suffered by the irradiated mothers, and in the morbidity, sex ratio and incidence of gross congenital malformations of the children. However, in 184 cases that were examined directly for ocular defects 4 out of 42 individuals irradiated *in utero* and 7 out of 142 children conceived after irradiation were found to carry severe ocular anomalies such as coloboma, atrophy of the iris, choroidal coloboma, strabismus etc. Although

no statistical significance in the incidence of such conditions could be demonstrated between the two groups and the doses delivered to the foetus were not known, 11 cases of eye malformations in a small sample of 184 subjects were thought to be an unusually high number, and the authors could not rule out the possibility that irradiation *in utero* (or before conception) might induce other and more serious malformations of the eye, in addition to heterochromia.

D. OTHER EFFECTS

1. Animal data

221. A number of papers deal with the damage of haemopoietic tissues during the foetal stages. Many of the earlier reports on foetal irradiation of various animals (11, 139, 295) refer to disturbances of haemopoiesis shortly after irradiation. Among the more recent contributions, Reincke (252) showed that rat embryos receiving 270 rad on the 18th day of pregnancy had a lower peripheral blood count which was at its minimum 8-12 days after the irradiation. This was followed by an over-recovery with a peak at 50 days, the haemopoietic regenerative capacity of the surviving animals being similar to that of normal adult animals. Rats irradiated 15 days p.c. with 120-240 rad showed a significant decrease of the white blood cell count (WBC) 1 day after birth. The haematocrit and the red blood cell count (RBC) were, on the other hand, unaffected (117).

222. In the case of mice exposed 15 days p.c. to 50 and 100 R, the WBC at day 17 p.c. was higher than controls, while the RBC was lower. This response is in contrast to the adult reaction. The platelet level was also depressed, but all the haematologic values returned to normal by the third week of age (285).

223. These data regarding the peripheral blood picture, as well as the data on the intrinsic radiosensitivity of the foetal haemopoietic precursors (323), show that the reaction of the foetal haemopoietic cells is compatible with the available information on sensitivity and cell kinetics of the blood-forming system of the rodent. These data are relevant to the post-natal survival of irradiated animals, discussed in paragraphs 181-195, and confirm the transient character of the haemopoietic changes induced by sublethal doses of radiation. The data of Christensen and Jackson (64) would imply, on the other hand, that the fraction of irreparable damage to the haemopoietic system of the foetal animal is higher than in the adult.

224. Kusama and Yoshizawa (165) studied the effects of ^{137}Cs gamma radiation (5-100 R) on the mitotic index of the ddY mouse foetal liver cells (14-16 days p.c.). Following an analysis of the dependence on time post-irradiation of the cellular changes at the various doses tested, they concluded that the threshold exposure in regard to the decrease of the mitotic index and to the frequency of prophase cells was between 5 and 10 R. Similar conclusions were reached by Hoshino *et al.* (126a) in an analysis of the mitotic delay as a function of radiation exposure in the matrix cells of the mouse telencephalon at 13 days p.c.

225. Studying the effects on male fertility, Rugh and Jackson (283) observed a 45-per-cent reduction of litter production in mice exposed to 200 R at 15.5 and 16.5 days p.c. Rugh and Wohlfromm (288) reported on the effects of lower exposures (25 or 100 R) at 0.5-18.8 days p.c. The male offspring were tested for their reproductive potential when their ages were 2-8 months. In contrast with irradiation during the embryonic stages, where some reduction of the reproductive potential was observed, the doses used during the foetal stages were insufficient to produce any reduction of fertility.

226. In females, Langendorff and Neuman (166) showed that exposure for 5 consecutive days to 20-80 R per day during blastogenesis, the first and second half of organogenesis, or the foetal period reduced the frequency, size and reproductive capacity of the litters. The greatest reduction appeared in animals irradiated between 11 and 15 days p.c.; with exposure rates higher than 60 R per day the offspring had no further descendants. The number of litters produced depended on the dose and the phase of gestation at irradiation, the differences of the reproductive capacity depending more critically on the frequency than on the size of the litters.

227. Ershoff (84a) described the effects of irradiation on the development and morphology of the reproductive system of male and female rats. Male animals exposed to gamma rays (300 R) at the rate of 17.92 R/min on day 18 p.c. or exposed to the same dose at the rate of 0.03 R/min over the period 13-20 days p.c. had a significant degree of gonadal damage. Females, in contrast had normal-size ovaries following the acute exposure but significantly smaller ovaries and an absence of follicles and corpora lutea following the long-term exposure.

228. Testicular atrophy in adult rats exposed to as little as 50 R on the 18th day of their foetal life was also reported by Brent (29) and cited as an unusual phenomen of an organ showing two widely separate peaks of sensitivity at 9 and 18 days of pre-natal development. Very recent data of Erickson and Martin (84) have revealed important discrepancies between the rat and the pig in regard to effects on the germ cells following continuous exposure throughout gestation.

229. Further information about the effects on the ovaries of the foetal mouse and rat as a function of gestational age (8-15 days p.c.) and radiation exposure (50-100 R) can be found in Mintz (197), Beaumont (15, 16), and Beaumont and Mandl (17). Other data on the fertility and reproductive capacity of rats exposed *in utero* have also been reported by Murphree and Pace (212) and Baev *et al.* (10).

230. Erickson and Martin (84) reported experiments on the pig irradiated continuously for 108 days (the gestation period in this animal lasts 112 days) at dose rates of 1.5, 3, 9 and 20 rad per 22-hour day with a ^{60}Co gamma source. Foetal dose rates were 0.5, 1, 3 and 7 rad per day. At all dose levels the health of the mothers, the number of live births and their post-natal viability appeared to be normal. At birth and again at 70 and 150 days of age the irradiated offspring were tested for body weight and organ development. Weight and

growth of the body were unaffected by dose rates of 3 rad per day or less. At this dose rate only the weight of the brain was decreased, and at 1 rad per day or less only the weight of the gonads was affected. Sterility was observed both in male and female animals down to 3 rad per day, and an appreciable reduction of the germ cell number was observed in both sexes even at 0.5 rad per day. Parallel experiments conducted on the rat under similar conditions of chronic irradiation (21 days of a 22-day gestation period) revealed an important inter-species difference in that the dose of 1 rad per day did not produce apparent effects on the germ cell population in either sex.

231. Cataract formation in the mouse irradiated during the foetal stages has been described by Rugh, Duhamel *et al.* (270) and Rugh, Wohlfromm *et al.* (292).

232. Two monkeys (*Macaca mulatta*), irradiated during foetal development (200 R on day 80 p.c. and 300 R on day 60 p.c.), were followed for 23 months of their lives. The studies covered the changes in the electroencephalographic, electrocardiographic and electroretinographic recordings. The brain appeared functionally impaired, concomitantly with signs of microcephaly. Depression of light reactions documented a reduced retinal function, while electrocardiographic changes were relatively minor (274).

233. Slightly but distinctly higher levels of urea nitrogen were detected in the blood of beagle dogs irradiated with 20 or 100 R *in utero* 55 days p.c. (341). In the interval 270-435 R of ^{60}Co radiation (delivered at the same time or soon after birth) high incidences of renal failure were reported, particularly in male animals (246).

2. Human data

234. Data have also been reported concerning the haematology of humans exposed *in utero* at Hiroshima (340). The sample size varied during the period of observation, 1951-1958, totalling up to 1020 exposed children and an adequate number of controls. The sample was divided by year of examination, gestation time at irradiation, sex, and distance from the hypocentre. Little indication was given of the actual doses received, apart from the remark that the exposure beyong 2 km was probably less than 20 R. No changes peculiar to the irradiated group were observed. A homogeneous subsample of 63 children, exposed during the first semester of gestation, was analysed. No correlation was found between leukocyte, erythrocyte and haemoglobin values and dose, since the changes associated with distance were comparable with the variation within each distance group. The progressively decreasing count of total leukocytes with age, which qualitatively is a common finding in man, appeared to be faster than normal, but this fact could not be attributed with certainty to any specific cause.

235. Haematological findings in adolescents exposed *in utero* at Nagasaki were also negative (47), in spite of a good assessment of the doses to the mother. It may therefore be concluded that, whatever haematologic changes might have taken place soon after exposure, there was complete haematological recovery by the age of 5 years. Furthermore, the profound changes of endocrine activity during adolescence did not bring to light any haematological deficiency in the exposed individuals.

236. It has been reported that parasitic infestation occurred at a significantly greater rate in children whose mothers were within 2 km of the hypocentre, a finding which, in the view of the authors, is probably to be associated more with dietary, living and socio-economic habits than with radiation (47).

237. Determination of butanol-extractable iodine in serum performed on 249 15-year-old children, some exposed *in utero* at Nagasaki and some unexposed, revealed no statistically significant differences between them with respect to dose and trimester of gestation at irradiation (49).

238. The urine analyses of children (9-16 years old) exposed *in utero* at Hiroshima or Nagasaki were compared with a suitable control group (90). An increased proteinuria was detected at various ages in Hiroshima boys and in all girls whose mothers were within 1.5 km of the hypocentre. Although in some of the groups the differences reached significant levels, the biological significance of these findings remain to be explained.

239. Two conflicting reports are available concerning the sex ratio of babies born from mothers exposed *in utero*. These reports should be examined with the purpose of analyzing the response of germ cells as a function of their stage of development at the time of exposure. It is known that in the human female the oogonial divisions stop about 5 months p.c., when all cells enter meiotic prophase. Meyer *et al.* (190, 191) reported on the sex ratio in children from a group of black women born in Baltimore in 1947-1949, about one third of whom had been exposed to x rays *in utero* in the course of diagnostic pelvimetry. No precise estimates of the radiation doses were given, but they may be assumed to be 1-5 rad in most instances (133). Each exposed person was matched with two controls by hospital of birth, parity and birth data. By 1966, 1993 members of this population had given birth to children and had experienced a number of foetal and neo-natal deaths and abortions. The rates of these events were indistinguishable in the exposed and control groups. A number of variables related to pregnancy, delivery and clinical history of the babies were also identical, showing that matching had really resulted in comparable groups of exposed and unexposed mothers. Under the conditions of exposure examined, it clearly appears that the reproductive capacity of the individuals had not been affected, at least up to 17-19 years of age. However, when the exposed group was subdivided as a function of gestation time at irradiation, it was possible to show that mothers exposed early (0-29 weeks) in their own foetal life had an unusually high and statistically significant excess of male babies. This suggests, in the view of the authors, an excess of pre-natal loss of female conceptuses, which might occur as a result of non-disjunction in the irradiated oocytes, because XXY males would be 10-30 times more viable than XO females.

240. The conclusions reached by Meyer *et al.* (190, 191) are indeed difficult to reconcile with the data of Jablon and Kato (133) based on the experience of Japanese A-bomb survivors. No effect on the sex ratio of the offspring was found, irrespective of the dose group or the trimester of gestation at irradiation. The data were completely negative, despite the larger radiation doses and sample sizes involved in the Japanese series. The question of possible alterations in the sex ratio of children born to parents which had been exposed *in utero* must therefore remain open for the time being.

241. Another epidemiological study was published recently (192) concerning 1458 black women exposed *in utero* to diagnostic doses (1-5 rad) of x rays and the same number of carefully matched controls. The reproductive patterns of the two groups were investigated as a test for possible injuries to the ovaries and oocytes irradiated during foetal life. To this end, the number of live births and foetal deaths in the offspring of the two groups were ascertained and compared. A 10-15 per cent increase in the fertility of exposed women was found. It was statistically significant ($P = 0.011$) and remained so even after adjustment between exposed and controls for socio-economic and medical factors. There was some evidence that this higher fertility might be decreasing with time and that a parallel rise in the rates of spontaneous abortions and foetal deaths might occur in the exposed group. If this trend is confirmed, it is possible that the total reproductive capacity of the exposed individuals may eventually be less than that of the control sample. A prevalence of other minor health problems was also found in the follow-up of the exposed women.

242. In a publication by Blot *et al.* (25), various reproductive indices were determined up to 1973 for a cohort of 2457 persons exposed *in utero* to the A-bombs in 1945 and for another 63 persons in a different sample who had a small head-circumference. It was found that the marriage rate was significantly lower in persons heavily exposed *in utero* (more than 100 rad maternal dose) than in unexposed or more lightly exposed individuals. This difference was attributed in part to the lesser marriage ability of the mentally retarded, who were significantly more numerous among the heavily exposed, and in part to other unknown variables, including possible social discrimination against survivors of the bombing. However, among those married, the frequency of childless marriages, the number of births and the interval between marriage and first birth showed no consistent relation with exposure.

V. INTERNAL IRRADIATION

A. GENERAL

243. The first reported observations on the effect of injected radioisotopes in the foetal mammal are those of Bagg (11). He injected radon solutions in amounts of tens of millicuries into pregnant rats at different gestation times and described post-implantation death and various types of malformations in the offspring. More recently, some data on teratogenic effects of internal irradiation have been published, but information on any one nuclide is still very scanty. It does not seem possible to draw general conclusions, and the main value of the existing data is the indication of possible levels of toxicity for the various radioisotopes. The available data will be reviewed according to the nuclide tested, but only for those nuclides for which there seems to be significant information. It was felt that the present state of knowledge in this field would not justify any attempt to express the data in terms of the radiation dose actually received by the conceptus or its organs and tissues. Exposure or dose data are therefore given according to the way they were reported in the original publication.

244. Two documents have been submitted to the Committee summarizing the effects on foetus and progeny of mothers exposed to radionuclides before conception (177) and reviewing the various effects in animals treated with radioactive substances in the course of intra-uterine development (178); both of them dealt with work carried out in the USSR. An indexed bibliography dealing specifically with the transfer through the placenta and into the foetus of radioactive substances injected to mothers is also of interest for reference to published data on this subject (335). The emphasis of the presentation to follow, in accordance with the objects specified in the introduction, will however be centred on the teratological effects themselves induced by different doses of the administered nuclides, rather than on the mechanisms and rate of transfer of such nuclides from the mother to the foetus. For these the reader is referred to the specialized literature included in the previously mentioned publications.

B. TRITIUM

245. Concerning the passage of tritium administered under the form of tritiated water from the mother through the placenta and into the foetus, Moskalev *et al.* (201) reported that placental accumulation depended only very slightly on the gestational age at the time of treatment; it was found to be 0.38 per cent at 15 days and 0.45 per cent at 19-21 days of pregnancy. In the foetus, 0.38-0.40 per cent of tritium was found on day 13-15 p.c. and 2.0 per cent on day 19-21. Istomina *et al.* (132) studied the transfer of tritium from the mother to the offspring by milk. Lyaginskaya (176) investigated the relationships between the pregnancy stage at the time of treatment with HTO in the rat and the ensuing effects in post-natal development. For a dose of 0.3 μCi/g, little relationship was found among these variables, although treatment at implantation did cause a somewhat increased pre-natal death of the embryos and administration during the foetal stages resulted in greater post-natal death. However, at this dose no teratological effects were manifest. LD_{50} values for foetal mortality in rats after single HTO treatments at various doses were 0.1 μCi/g (corresponding to an estimated dose to the foetus of 100 rad) at 4 days p.c.; 10 μCi/g (400 rad) at 9 days p.c.; and 100 μCi/g (1000 rad) at 17 days p.c. (178).

246. Cahill and Yuile (53, 54) evaluated the resulting foetal tissue doses in rats, the body water of which was

maintained throughout pregnancy at a constant tritium level in the range 1-100 μCi/ml. This was achieved by adjusting the ingestion of tritiated water. Dose rates in embryos and foetuses were calculated to range from 0.3 to 30 rad per day. Most mothers were sacrificed before birth for observation of the conceptuses, but in some cases the observations were carried out on born offspring and followed to their adulthood. Several statistically significant effects were found at various HTO levels, in no apparent relationship with dose. These included microcephaly, sterility, stunting, reduction of the litter size and increase in the resorption frequency. Stunting appeared at 20 μCi/ml and its degree increased at higher concentrations in direct relation to dose. Organ weight decreased in proportion to dose. The incorporation of tritium into foetal organs was directly proportional to the maternal HTO activity during gestation and amounted to 20-30 per cent of this activity. After 180 days of life, stunting only persisted in males with concentrations in the range 50-100 μCi/ml.

247. A paper by Dobson (75) dealt specifically with the RBE of tritium relative to ^{60}Co gamma radiation in long-term exposures. To this end, tritiated water in various doses was administered in drinking water to pregnant mice throughout gestation and lactation for a total of 33 days, the specific activity of the body water being checked by the radio-assay of urine samples. Other groups of mice were exposed to ^{60}Co gamma radiation at various dose rates for the same time lapse. The number of oocytes in the progeny of these animals was counted at the end of treatment, and it was found that oocyte survival decreased exponentially with tritium concentration in body water with an LD_{50} level of about 2 μCi/ml body water, corresponding to an effective dose to the foetus and the new-born offspring of about 6.5 rad. The survival curve of oocytes with respect to ^{60}Co gamma rays was convex upward, indicating a decreased killing effectiveness of the gamma-ray treatment at low doses. By comparing individual gamma-ray and tritium experiments it was shown that tritium was more effective, and limiting RBE values were found to vary between 2.5 and 4.2 with a likely maximum value of about 3 at doses approaching zero. Short-term and protracted exposures were also compared by the same end-point, and higher RBEs for protracted irradiations were obtained.

248. Recent attempts were made by Cahill *et al.* (55) to assess by a number of different end-points (morphological, biochemical and functional) the effects on rats of a simultaneous long-term administration of lead and tritiated water. The experiments allowed the important general conclusions that combination of the treatments resulted in less-than-additive effects and that a significant reduction of brain weight by tritium exposure was apparent at dose rates of the order of 300 mrad per day continuously from conception to 180 days of age. Haemopoietic disturbances (anisocytosis, leukopenia, thrombopenia) in the progeny of rats given tritiated water at doses of 0.008-0.3 μCi/g were described by Zhukova (reported in (178)). Animals from mothers treated at 0.3 μCi/g on day 4 p.c. were the most severely affected. Finally, Lyaginskaya (178) reported a decrease in the life-span of three subsequent generations of rats irradiated *in utero* with tritiated water on day 4, 11 and 17 p.c., particularly upon treatment on day 4 p.c.

249. Concerning administration of tritium in the form of tritiated thymidine, it has been possible to study a number of effects on both the mother and the foetus induced by a continuous perfusion of tritiated thymidine (^{3}HTdR) to pregnant rats from day 9-22 p.c. (88, 101). Activities of 1.6 μCi/g per day were in general well tolerated; but higher levels, from 3.2 to 6.4 μCi/g per day, produced a marked bone-marrow syndrome in the mother. Although the litter size at birth did not appreciably change at these levels, the percentage of still-born offspring increased with the activity injected, while the number of offspring surviving more than 12 hours after birth decreased in proportion to that activity. With 8 μCi/g per day, no rat was born alive. Retardation of growth and macroscopic and microscopic malformations of the head, brain, eyes, ears, mouth and extremities were observed at these high levels. With 6.4 μCi/g per day, no gross external abnormalities were seen, but the general development and the haemopoietic system of the animals were severely impaired in proportion to the administered activity. The post-natal growth and weight of surviving animals also showed activity-related pathological changes.

250. In another paper (311), the incorporation of tritium from ^{3}HTdR in developing rats was studied by biochemical techniques. The total incorporated activity and the DNA specific activity showed a direct relation to the activity of the tritium injected in the mother. The distribution of radioactivity between the DNA and the low-molecular-weight fraction was independent of the administered activity, and the time variation of the DNA specific activities was characteristic for each organ. Because of all these facts, the system of continuous perfusion was recognized as a suitable procedure for studying ^{3}HTdR toxicity in the embryo.

251. Mouse embryos were also grown *in vitro* from the 2-cell stage to the blastocyst stage in the presence of ^{3}HTdR (329). Concentrations of tritium above 0.1 μCi/ml were definitely lethal, and concentrations between 0.01 and 0.1 μCi/ml caused a highly significant reduction of the number of cells in the blastocyst. The latter effect could be largely accounted for by selective cell death occurring at the 16-cell stage. As the cells in the inner mass were most susceptible to killing, it was possible to obtain blastocysts composed entirely of trophoblast.

C. PHOSPHORUS-32

252. Burstone (50) described the effects of ^{32}P on the development of embryonic tooth primordia in mice, showing that injections of 5-17 μCi/g in pregnant animals 2-6 days before birth gave rise to developmental disturbances of teeth, observable within about one month after birth. He also reported that activities above 10 μCi/g produced general post-natal growth retardation and that this effect was more evident when ^{32}P was given early in pregnancy.

253. Sikov and co-workers carried out a series of systematic quantitative studies on the consequences of ^{32}P irradiation throughout gestation. Sikov and Noonan (320) gave several IV injections of the nuclide to female

rats on day 6, 8, 9 and 10 p.c. They then established the values of the LD_{50}, for lethality of the foetuses before day 14 p.c. These LD_{50} values increased with the age of the embryos at treatment, from 0.46 mCi on day 6 p.c. to 1.29 mCi on day 10 p.c. The uptake of the isotope by the foetus was assessed, and from this the doses to the embryos were calculated for each day of gestation. At the LD_{50} levels mentioned above, the doses varied from about 6000 rad on day 6 p.c. to about 10 200 rad on day 10. The uptake appeared to be proportional to the activity injected, indicating that there was no major interference with the phosphorus metabolism. A reduction of embryonic weight was found at the time of observation, the reduction being greater when embryos were irradiated earlier.

254. Growth retardation (321) and skeletal defects (316, 317) were also studied in rats, following the administration of 0.6-2.0 mCi of ^{32}P on days 14 and 17 p.c. The study of specific defects and the measurements of various bones were carried out using serial sacrifice from injection to birth. A dose-related decrease of the foetal size and of the bone sizes was observed and this decrease was found to be greater in animals tested at the younger developmental age. The same pattern applied to skeletal malformations, which were also more frequent and severe in the group injected on day 14 p.c. Differences with respect to previous experience with x irradiation were noted (357). With the aid of a dosimetric study (314), such differences could in part be attributed to the pattern of accumulation of the radiation doses at the two ages tested. Other possible explanations related to the effects of the dose rate or of the LET of the radiations compared (315).

D. STRONTIUM-89 AND STRONTIUM-90

255. Most of the early papers on the effects of ^{89}Sr and ^{90}Sr dealt with the transfer of the nuclides through the placental barrier (244, 360, 124, 221). Finkel (86) concluded that, in the mouse, negligible amounts of ^{89}Sr were transferred through the placenta during the first 15 days of gestation. She described an increase in the percentage of still births, some growth retardation, bone fragility and anaemia in mice treated *in utero*. In the rat, the retention of ^{90}Sr in the placenta was estimated to be 0.02 per cent of the amount injected and was found to be rather independent of the gestational age at treatment. However, the passage of the nuclide to the foetus was such, that for early gestational ages its content equalled that in the placenta (0.01-0.02 per cent) but at 16-19 days was about 20 times higher (201). Hopkins and Baxter (125) reported that the fraction of the ^{90}Sr injected into pregnant female rats at 17 days p.c. which was retained at birth by the offspring decreased with increasing maternal age at injection, although the ratio of offspring body burden to maternal content was independent of maternal age under the specific conditions tested. There was no marked difference in the percentage of the dose of ^{90}Sr retained by new-born offspring in the dose range 24-382 μCi.

256. Single injections of ^{90}Sr to pregnant rats at various dose levels were used to establish the values of LD_{50} in the offspring. They were found to be 0.4 μCi/g (corresponding to a calculated foetal dose of 3.5 rad) at 4 days p.c. and 0.8 μCi/g (10 rad) at 15-19 days p.c. (data reported in (178a)). A dose of 10-15 μCi/kg of ^{90}Sr injected IV in pregnant dogs at 1-9 days before birth produced no obvious malformations (87). Burykina *et al.* (51) concluded that in their experiments the chronic introduction of ^{90}Sr (0.02 μCi/kg per day in the course of 3-3.5 years) produced skeletal doses of about 50 rad/year in the parents. Under these conditions, disturbances in the development of the progeny were observed, together with a decrease of their viability. A reduction in the number of oocytes was described by Nilsson and Henricson (224) in mice after IV injection of the pregnant females with 20 μCi of ^{90}Sr. The effect was more pronounced with injections at day 16 than at day 11 p.c. Finally, Kincade *et al.* (147) described changes in the haemopoietic and immunopoietic cell populations of the foetal liver, marrow and spleen after ^{90}Sr treatment.

E. IODINE-131

257. After single parenteral injections of ^{131}I (4 μCi/g) at 1-16 days p.c. in the rat, pre-implantation embryos were found more susceptible than those at later stages; for treatment at day 1 p.c. a three-fold increase in foetal intra-uterine mortality compared with controls was obtained (164). Oral administration of 1.0 μCi of the same nuclide on day 13 produced an accumulation of 0.035 per cent in the placenta and 0.01 per cent of the administered activity in the foetus. By day 20 or 21, the amount in the placenta was increased only slightly while the foetal retention was 28-40 times that at day 13. This increase was related to the increase in the mass of the foetal thyroid and to an improvement of its ability to concentrate the isotope (179).

258. Chronic treatment at the level of 65 μCi per rat daily throughout pregnancy led also to a significant increase of the death rate (about 41 per cent), of which 10 per cent could be accounted for by pre-implantation, and 31 per cent by post-implantation, mortality. Delay in the development of the progeny, microcephaly and liver damage (chromosomal aberrations) were also apparent (164). Iljin *et al.* (data in 178a) also reported on repeated treatment of rats with ^{131}I at 1-7, 8-14 and 14-21 days p.c. for a total of about 30 μCi per rat given at progressively lower doses. According to the various treatment schedules, an increase in mortality at birth of about 15-25 per cent was seen. A delay in thyroid development was also apparent. Finally, histological changes in adult mouse thyroids following treatment of the mothers with ^{131}I at 18 days p.c. were described by Walinder and Sjödén (352) but no reference was made to teratogenic effects induced by the treatment.

F. OTHER NUCLIDES

259. Female rats were exposed to monomeric or polymeric ^{239}Pu on day 15 or 19 p.c., and the distribution of the nuclide in various portions of the foetoplacental unit was studied by counting and by autoradiography (319). At the above gestational ages, 50 μCi produced no pre-natal mortality. On the other

hand, a substantial difference in the mortality at 14 days following an injection on day 9 p.c. was observed; 2-12 μCi induced about 60 per cent mortality and 25 or 50 μCi produced death of all embryos. None of the surviving foetuses showed gross morphological malformations. In view of the high activities administered, the value of these experiments is purely theoretical and without applicability to risk estimation.

260. In order to establish the $LD_{50/30}$ of ^{241}Am for the rat foetus at various stages of development, the pregnant females received IV injections of the nuclide at 10, 13, 16 and 19 days p.c. The concentrations of the solutions were increased stepwise from 1.2 to 7.6 per cent to cover the required range of doses, while the volume injected was kept approximately constant. Since the placenta behaved like an inconstant barrier (the ratio of the maternal to foetal concentrations of ^{241}Am varied from 6:1 to 2:1 (201)), the LD_{50} values were expressed as the doses to the mother that produced 50-per-cent mortality of the foetuses at different ages, followed by the calculated cumulative rad doses to the foetuses: 10 days p.c., 0.03 μCi/g (100 rad): 14 days p.c., 0.01 μCi/g (800 rad): 19 days p.c., 40.2 μCi/g (1000 rad) (375).

G. CONCLUSIONS

261. It seems impossible at present to draw any precise, general conclusions about the action of radionuclides in the development of the conceptus because of the paucity of data on dose-effect relationships for the nuclides tested. None of the effects observed up to now appears to be specific to internal irradiation, and these effects may be viewed as the end-points of a continuing action exerted during the various stages of embryonal and foetal life. Their precise interpretation and evaluation must be based on a precise knowledge of the dose accumulated by the mother and the foetus at any particular stage and in any particular sensitive structure under all possible conditions of administration. The information we have at present is far from being good enough for that.

262. In spite of some attempts to compound the known data on the placental transfer of the various nuclides into a general model of transport and accumulation (242, 332, 333), the information is still too fragmentary, not only with respect to the dosimetry, but particularly in regard to the effects. Careful consideration of the data reviewed allows only a schematic enumeration of the main variables that have been shown to interact in the production of the end-effects. They are *(a)* the nature of the radionuclide in respect to its physical characteristics and to the different organotropic behaviour; *(b)* the chemical form in which the nuclide is administered, in so far as it may influence its pattern of accumulation and deposition; *(c)* the route of administration and the dosage schedule, particularly because the rate of passage and accumulation from the mother into the conceptus may be critical in relation to the rate at which the ontogenic events take place in a rapidly-growing and differentiating organism; *(d)* the developmental age at irradiation, since periods of maximum sensitivity are to be expected in the case of internal as well as for external irradiation, even though they may be difficult to resolve owing to the continuous action of the deposited nuclides; *(e)* the animal species, not only in regard to the peculiarities of the differentiation pattern, but also in respect to all physiological differences in the absorption and deposition that could alter the incidence of the biological end-points; *(f)* the age of the pregnant animals at treatment, for which information is virtually absent; *(g)* the possible role of the maternal irradiation. Further extensive experimentation in all these fields, at least for the most hazardous nuclides, is clearly called for.

VI. MECHANISMS OF RADIATION TERATOGENESIS

263. The present chapter has two purposes:

(a) It tries to identify some common features and trends among the data and effects described. This information may explain the origins of malformations and the likely pathways involved in their manifestation;

(b) It discusses the scanty information available on the role of some radiobiological variables (LET, dose fractionation etc.) and of some of the modifiers of the teratogenic response (oxygen effect, chemical protection etc.).

264. Since the mechanisms of the lethal action *in utero* have already been discussed (paras. 66-70, 94-97, 186-191, 221-224), the discussion will be limited essentially to the induction of growth disturbances and malformations. A few remarks about the applicability to the human case of data obtained experimentally in animals are also included.

A. INFLUENCE OF THE MATERNAL ORGANISM

265. One issue concerning mechanisms which has attracted much attention, particularly in the older literature, is the influence that the irradiated maternal organism might have on the appearance of foetal abnormalities. The reasons for such a continued interest can hardly be explained, since it stems mainly from experiments of doubtful significance and it is known that malformations can also be induced by irradiation in oviparous species. The close relation of the conceptus to the mother in mammals is not by itself a sufficient reason to postulate any abscopal mechanisms. In a critical evaluation of the data, Russell (295) observed in 1954 that two facts stand against such a hypothesis: *(a)* the existence of sharply defined periods of sensitivity in the embryo, which cannot be easily reconciled with the relatively long-lasting effects of radiation in the mother, and *(b)* the extremely short time between irradiation and the appearance of embryonic lesions, which would require the improbable existence of very rapid mechanisms.

266. Among the more recent contributions, Chang and Hunt (60) have reported experiments in which pre-implantation rabbit embryos irradiated *in utero* were transplanted into non-irradiated recipients or into animals exposed to 400 R at different times after an

induction of ovulation by hormonal treatment. Under these conditions, embryonic mortality was increased by the irradiation of both the ovum and the mother, but the relative contribution of the two factors was found to depend on time elapsed since ovulation. Brent and Bolden (32) raised an objection to these experiments on account of the comparatively high doses used, which might actually have resulted in an increased foetal mortality (though not necessarily in an increased yield of malformations). They also criticized the lack of synchronization between development of the embryo and of the decidua, which is critical for proper embryo implantation. Experiments similar in many aspects to those of Chang and Hunt (60) were reported at the same time by Issatchenko (131) who transplanted 2-day-old rabbit embryos from irradiated animals (250 R) to normal females and *vice versa*. Under these conditions an increased mortality of the embryos was observed above that obtained by irradiation of the embryo alone, thus confirming the presence of an indirect effect of the irradiated maternal organism.

267. Duplan and Monnot (79) irradiated pregnant mice (200 and 400 R of x rays) with shielding of the foetuses and observed a dose-related increase of pre- and post-natal mortality in the offspring. Also, protection of the mother's abdomen resulted in a higher percentage survival of the non-irradiated offspring when compared with the shielding of the head and neck. Valentini and Hahn (349) studied the survival of rat embryos in the right uterine horn irradiated with 200-400 R at day 1.5 or 4.5 p.c. and found a significant increase when the left lower body quadrant was concomitantly shielded. These results would indicate that irradiation of the mother might be a contributing factor in pre-implantation embryonic mortality.

268. A series of papers by Brent and co-workers have contributed greatly to clarifying this issue. These authors were able to show that exposures of 200 R in rat embryos at 9 days p.c. were definitely lethal (38). When the embryo was shielded while the mother was exposed to x rays at 400 R of whole-body irradiation or to over 1000 R of partial-body irradiation, no embryonic malformations were seen in embryos at term. Foetal mortality, on the contrary, was higher than in control animals, presumably as a result of radiation sickness in the mother. When the placenta was exposed to 400 R (28), while both the mother and the conceptus were shielded, there were no lethal effects, growth disturbances or induction of malformations.

269. In another paper dealing with pre-implantation embryos (32), Brent and Bolden used shielding techniques to differentiate the results of ovary, oviduct, uterus and zygote irradiation (150 R). Treatment on day 1 p.c. showed that uterine and ovarian irradiation had no effect on foetal mortality, which instead was caused by irradiation of the oviduct containing the fecundated ova. Malformation incidence was very low, in accordance with the observations reported in paragraphs 75-79. Although this experiment does not rule out possible effects of oviduct irradiation, it strongly suggests that the major and primary action is caused by direct irradiation of the ova. Brent and Bolden (34) showed also that embryonic rats in the first day of development irradiated with doses of 150 R were resorbed in 60-70 per cent of the cases when irradiated directly, while irradiation of the mother and shielding of the embryos did not appreciably increase the resorption level above control. The same experiment with a dose of 400 R resulted in a 25-per-cent resorption of the unirradiated ova, showing a moderate indirect effect at the higher doses.

270. It may be concluded that irradiation of the mother is likely to be of very minor importance for the induction of malformations, particularly at low doses. On the other hand, it is conceivable that mortality of the embryo and foetus may be indirectly affected by the irradiation of the mother, especially at doses of a few hundred rads.

B. NATURE OF THE PRIMARY DAMAGE

271. In the mid 1950s, Russell and her collaborators (295, 302, 303) presented a complete analysis of the nature of the primary damage which develops into teratological effects. In the more recent literature, there have been few systematic treatments of the subject, although some special subjects have been dealt with more frequently (318, 255).

272. The materials reviewed in the preceding sections of this report show that the developing embryo is composed of a variety of dividing, differentiating, interacting and migrating populations of cells, arranged in complex and ever-changing patterns. Unlike the adult, the embryo is characterized by the absence of cell populations in steady state, and it is this circumstance that may be related to the variable radiation response as a function of dose and time. Molecular embryology is beginning to elucidate some of the intracellular mechanisms of gene regulation and some of the possible intercellular interactions taking place in orderly sequence in the various embryonic cell lines. However, the occurrence of certain "macroscopical" events in developing embryos cannot yet be related with certainty to specific molecular changes (19, 12, 26).

273. Nevertheless, two qualitative features of embryogenesis are important in relation to the effect of radiation. The first is the sequential character of development, the occurrence of certain events appearing to trigger the initiation of other events taking place at later times. The second is the constancy of the timing of the events, which occur within rather narrow intervals of time from conception. The first of these features explains the peculiar character of the radiation-induced malformations, which often appear to be the consequence of an arrest of development at some rudimentary stage; the second explains the specificity of irradiation at certain well defined times in the production of given types of malformations.

274. Conceptually, the mechanism of radiation-induced malformation may be examined at different levels of biological organization: *(a)* the subcellular level, where the problems of concern are the distribution of primary events within the cell and the resulting changes in subcellular structures; *(b)* the cell as a separate unit

reacting to the lesions of its components; *(c)* the cell population level, including the damage and repopulation of the compartments originating from common precursors and also the relations with other cell populations; and finally *(d)* the developmental pathways that lead from the injury of single cellular precursors to the final malformative change.

275. The information available at all levels is extremely limited. For some of the issues discussed in the following paragraphs there is no experimental data at all, and therefore the problems can only be formulated in very general terms.

276. At the subcellular level, it seems quite likely that the primary events taking place in embryonic cells are qualitatively equal to those occurring in other cell types. The differences between embryonic tissues with respect to water content, oxygen tension, concentration and properties of radicals seem insufficient to justify important differences of the primary effects (309). Substantial knowledge has developed in recent years about the effects of radiation on DNA and the action of reparative enzymes in mammalian cells (5). This information is regarded as of great radiobiological importance because of the essential role of DNA in cell division and differentiation. However, there are no direct data in embryonic or foetal cells.

277. Direct quantitative data on embryonic or foetal cells and tissues are at the present time few and scattered. Those available concern changes in the synthesis of DNA (198, 183, 184, 185, 324, 311, 199, 53, 54), RNA (198, 183, 184, 185) and proteins (198, 183, 184, 185, 313, 85); the disturbances in the ontogenesis of particular enzymes or enzyme systems (351, 216, 215, 214, 233, 163, 94); the ultrastructural changes of cytoplasmic organelles (233, 258, 259, 62); the analysis of kinetic changes of specific cell lines or tissues (83, 186, 185, 84, 165, 40, 41, 42, 199, 126, 107, 234). The species differences and the non-systematic character of the observations in regard to irradiation time, dose delivered, heterogeneity of end-points etc. preclude the formation of any coherent picture of the fundamental pathways through which gross teratological effects become expressed.

278. It seems logical to postulate nevertheless that the observation in irradiated mammalian cells *in vitro* and *in vivo* apply as well to cells irradiated *in utero* (309). It is also reasonable to assume, as a first approximation, that damage to the nuclear structures of the cells should be preferentially considered. In this context, two possible mechanisms of damage of the genetic structures have been proposed as the initiating processes: gene mutation and chromosomal aberration.

279. The role of gene mutations (365, 363, 364) is probably insignificant, in view of the very low frequency of these events at doses shown to be teratogenic, and also because of the low degree of expression of these mutations in diploid cells (295). In analogy with observations made on irradiated somatic cells of the adult (5), it seems quite reasonable to assume therefore that the major component of cellular damage is attributable to chromosomal abberations (295). Chromosomal aberrations are likely to impair cell reproduction owing to the chromosomal imbalance of the daughter cells or possibly to mechanical disturbances of the mitotic process.

280. It is generally agreed that cell inactivation can be the common denominator of all types of malformations. Microscopical evidence of cellular damage is, in many instances, the first change observed in irradiated embryonic cells of the nervous system (258, 120, 121, 161, 237, 238, 239, 308, 21, 207, 144), the eye (162, 284), the skeleton (295, 210), the urogenital system (243, 121) and many other tissues.

281. This uniform pattern of cell reaction is consistent with the great similarity of the malformative response obtained in any given primordium or stage (295). It should, however, be reconciled with the observed selectivity of the damage, which appears to develop preferentially in certain structures at certain times.

282. Mechanisms of differential sensitivity to radiation may be invoked to explain selectivity. Furthermore, in accordance with what is observed in adult tissues, it is possible to postulate a different intrinsic cell sensitivity to inactivation in different phases of the mitotic cycle, and also a differential sensitivity of tissues having different kinetic structures. Repair phenomena at the intracellular level and repopulation of the tissue compartments may also contribute to the specific sensitivity of the embryonic primordia during certain phases of their development when cell division and differentiation are most active. The normal sequence of these cellular periods is strictly dependent on some unknown regulatory mechanisms, and indirect damage to these mechanisms may also operate in the induction of abnormalities.

283. The loss of reproductive integrity of a cell is reflected in the immediate descendants of the affected precursor cell. It is also conceivable that damage of a given structure may result in secondary effects on other structures or processes which are functionally dependent on the originally damaged precursor and which may, in turn, be additionally affected by the radiation exposure. It should not therefore be expected that the large variety of processes involved can be directly inferred from a simple analysis of the malformative end-point (303).

C. DOSE-EFFECT RELATIONSHIPS

284. In the absence of direct information about fundamental mechanisms it appears nonetheless possible to formulate simplified mathematical models and to test their compatibility with observed data on dose-effect relationships. Such an exercise has been carried out by Russell (303); the model is illustrated in the following paragraphs.

285. If it is assumed that all reproductive cells in a given embryological structure have, as a first approximation, the same probability p of being inactivated by a given radiation treatment, and if it is further assumed that p is a function of dose, the number of inactivated cells in the

structure will be distributed according to a binomial distribution of the type

$$P(x) = \binom{n}{x} p^x (1-p)^{n-x}$$

where $P(x)$ is the probability of encountering x inactivated cells, and n is the number of reproductive cells in the structure. It may also be postulated that there is a threshold x_T of the number of inactivated cells below which no teratological damage will be apparent. This threshold could be different for each structure and presumably related to its repair capacity. If the damage inflicted on the structure exceeds the value of the threshold, a malformation would be induced and its severity would be in proportion to the number of cells damaged above the threshold. The probability of inducing a malformation would then be

$$P = \sum_{x=x_T}^{n} \binom{n}{x} p^x (1-p)^{n-x}$$

286. Under these hypotheses, Russell (303) has shown that, irrespective of the value of n specifically assumed for each structure, a dose increase will produce an increase in both the number and the severity of the malformations. Furthermore, even with fixed values of p and n, owing to the variability introduced by cell division of the cells retaining their capacity for proliferation and of their descendants, some variability in the malformative end-point will be expected. This variability will operate even under conditions of good homogeneity of the genetic background of the animals and of the experimental conditions under which a given dose-effect relationship might be determined. Other causes of variability, discussed at length in previous sections of this Annex, will obviously add considerably to these inherent causes of variation.

287. It follows therefore that the derivation of the actual values of the parameters mentioned above through the simple mathematical analysis of measured dose-effect relationships would give highly uncertain results of purely speculative value. It would not be justifiable to interpret such data in terms of simple underlying mechanisms, even in the most simple case of an apparently linear response.

D. THE PROBLEM OF THE THRESHOLD

288. The frequently encountered sigmoid nature of dose-effect relationships for damage to the embryo and foetus (see chapter III, section C), the action of repair systems at the subcellular and tissue levels (paras. 271-283) and the theoretical notions to be gained by models of the type described in paragraphs 284-287 strongly suggest the existence of non-linear components in the dose-effect relationships or possibly the existence of true thresholds.

289. Two approaches have been followed in regard to such problems. One is the determination of the lowest doses at which various abnormalities have actually been observed (lowest teratogenic doses). The other consists of experimental tests aimed at establishing the existence of thresholds.

1. Lowest teratogenic doses

290. The literature contains many tabulations of the minimum doses at which different types of malformations have been observed in various experimental animals (27, 92, 370). Table 5 presents an updated

TABLE 5. SYNOPSIS OF THE LOWEST TERATOGENIC DOSES OBSERVED IN MICE AND RATS

Species	*Gestational age (days p.c.)*	*Exposure (R)*	*Effects observed*	*Reference*
Mouse	0.5	5	Increase in resorption frequency	278
	0.5-1.5	15-20	Exencephaly	276
	0.5-1.5	5	Polydactilia	277
	7.5	5	Increase in resorption frequency	231
	7.5	5	Skeletal malformations	
	7.5	5	Decrease in litter weight (winter sample)	136
	7.5	5	Hydramnios	
	7.5	5	Reduced tail length	
	7.5-8.5	25	Malformations of the axial skeleton	297
	8	25	Hydrocephalus, flexion of the spinal cord, architectural changes of ependymal cells	204
	8.5	50	Eye defects	181
Rat	0-8	5-25	Growth disturbances	27
	8	12.5	Growth disturbances	362
	8-9	36-40	Ocular and cerebral malformations	139
	9	25	Growth disturbances	365
	9	50	Increase in resorption frequency	364
	9	12.5	Ocular and cerebral malformations	93
	9	100	Heart and aortic, face, and urinary tract malformations	362
	9	50	Brain and spinal cord malformations	362
	9	25	Microphthalmia, anophthalmia	362
	16-22	10-40	Permanent alterations of nerve cells and cortical architecture of the brain	120

summary for the mouse and rat, in which species sufficiently low doses have been tested. Doses of the order of 5 rad, administered during the period of major organogenesis, have definitely been shown to induce malformations, particularly in the skeleton of the mouse, together with some lethal effects and growth disturbances. There appears to be no systematic investigations with rats at the same dose level, but it seems likely that for doses of a few rads, careful experiments might reveal teratogenic effects, particularly growth disturbances and ocular defects. The table also shows that in the nervous system, where cell division and differentiation proceed to very late foetal age, doses of a few tens of rads can produce microscopic lesions of the nerve cells and malformations of the cerebral architecture. The observation accounts for the fact that microcephaly and mental retardation can be induced throughout foetal life with comparatively high frequency (see paragraphs 141-146).

291. The fact that teratogenic effects have not been shown in humans with doses of the same order of magnitude in the course of pelvimetry or other diagnostic procedures (see paragraphs 202-208 and 217) could possibly be attributed to the developmental heterogeneity of the irradiated samples and to the late age at irradiation, which in the majority of cases was after the organogenesis period. It can be concluded, however, that in those animal species where low doses of irradiation have been thoroughly tested at the time of the sensitivity peak, there is evidence of some effect at doses as low as 5 rad.

2. Experimental tests

292. Much of the work concerning the experimental demonstration of threshold is insufficient to permit drawing definite conclusions. The usual reasons are the small number of animals used, the absence of wide-range dose-response data, the relatively high doses employed and, in some cases, technical imperfections. Threshold problems have been particularly considered by Jacobsen (136). His experiments concerned skeletal abnormalities induced in 7.5-day-old mouse embryos by exposures of 0, 5, 20 and 100 R. The data are limited to one type of malformation and to a single embryonic age (in order to improve the precision of the estimates), but the limitations are largely compensated by the number and accuracy of the observations. However, it is impossible to predict to what extent his conclusions would apply to other malformations and embryonic ages.

293. Jacobsen concluded that dose-effect relationships for single malformations are of little practical relevance for an overall evaluation of the teratogenic effects, mainly because of the low frequency of occurrence of such events. He therefore grouped the scored malformations according to skeletal region. The effects were found to be linear or almost linear with dose, within the confidence limits, for the control, 5-R and 100-R groups, while the 20-R group was in most cases slightly below the expected value. This pattern was found to apply to most of the skeletal regions examined and also to other traits not involving the skeleton. It was found in summer as well as in winter experimental samples.

294. In spite of the slight departure from linearity, however, the author concluded that there was no suggestion of threshold, since both the 5-R and 20-R points were significantly above the values of control. Clearly these experiments do not exclude the possibility that thresholds might exist at exposures below 5 R, but experiments at lower doses would require a much greater number of animals and result in having, in principle, the same problem, but within a lower dose range.

295. It may be concluded that the most refined experiments so far exclude the existence of threshold for exposures as low as 5 R at least under the specific conditions tested. The low-dose effects (at 25 R or below) which have been recognized in animal experiments (136, 204, 231, 297) may be regarded as an increased incidence of some types of malformations or effects occurring spontaneously in the species tested. It may be very difficult to establish threshold doses for these effects, since the incidence of spontaneous malformations and minor anomalies is controlled by the genetic constitution of the strain or of the individual, which has an intrinsic variability.

E. FACTORS MODIFYING THE TERATOGENIC RESPONSE

296. In addition to the factors examined systematically in the other sections of the Annex with regard to each type of malformation, there are other factors that can modify the teratogenic response to a given radiation dose. These include physical factors such as LET and dose rate and the modifying effect of oxygen and chemical protectors.

1. Radiation quality and LET

297. The type and energy of radiation, as reflected by the ionization density and the LET, are among the most important radiobiological parameters affecting the degree of response (256). There are, however, very few experiments on LET effects on the induction of malformations in mammalian embryos. Furthermore, these experiments are not systematic and, in particular, the data do not include the effect of a range of LET values for various abnormalities at different embryological ages.

298. Some very early reports (11, 52, 106) are hardly amenable to quantitative analysis. Sikov and Lofstrom (315) reported on the RBE of ^{60}Co gamma rays and 250-kV x rays in rats irradiated at various gestational ages (9-10.5 days p.c.). The exposures (110-450 R) were delivered either acutely every 12 hours or intermittently throughout most of the half-day interval between exposures. Embryos were examined at 14 days p.c.: mortality and malformations of the mandible, eye, maxilla, extremities and tail were considered. It was found, in general, that gamma radiation produced less effect than x radiation, giving rise in most instances to RBE values in the range of 0.80-0.95. However, for malformations of the eye, maxilla and mandible induced at 9.5 days p.c., substantially lower RBE were obtained. The slopes of the dose-effect curves for mortality were

the same for both radiation schedules used, regardless of embryonic age and protraction. Concerning malformations, similar dose-effect curves were produced by the two radiations but sometimes protraction resulted in curves of smaller slope; a reduced magnitude of some effects and an increased value of the RBE were induced by protraction. The effects were interpreted as depending on the differences of recovery rates of embryonic structures, especially when the change of RBE by fractionation was particularly evident.

299. Friedberg *et al.* (91) irradiated mouse embryos in the pronuclear-zygote stages with 300-kV x rays (67 rad/min) or with fission neutrons (2 rad/min) and examined them for embryonic survival of the uterine content of the mother 16 days later. Non-threshold exponential dose-survival relationships were found to apply to both x rays and neutrons in the range of 0-100 rad and 0-19.5 rad, respectively. A comparison of the slopes resulted in an RBE of 4.5. With increasing dose, the pre-natal mortality increased, and the embryos died at earlier development stages.

300. These data are clearly insufficient to allow generalization, although they appear to be compatible with our knowledge of RBE for other somatic effects in mammalian systems. More systematic investigation for other radiations and LET spectra are required to cover the subject adequately.

2. Dose rate and fractionation

301. In contrast to the paucity of data on the effects of radiation quality, there are comparatively numerous studies on the effects of dose rate and fractionation. The reason for this interest is the possibility that protraction might not reduce the effect in a system like the embryo. In view of the complex pattern of cell types, each with a different time-related pathway of division, differentiation and interaction, a protracted dose could in principle produce more adverse effects in the embryo than an acute dose of the same magnitude. However, it should be pointed out that it is extremely difficult to control these experiments, mainly because of the rapid changes in sensitivity taking place as a function of time in the various structures and of the changing pattern of malformations specific to each developmental period.

302. The report by Auerbach (7) did in fact show some increased hazard in mammals of fractionated, as compared to single-dose, irradiation. Hybrid mouse embryos at 9.5 days p.c. were exposed to either 300 R or to three 100-R fractions delivered at 30-min intervals and were examined by uterine dissection 3-6 days after irradiation. The criteria of damage used were the incidence and degree of lumbosacral spina bifida and coloboma of the eye. While the total incidence of malformations was not affected by fractionation, their severity increased: this effect was seen in both abnormalities, in spite of their different developmental pathogeneses.

303. The observations of Rugh and Grupp (279) covered the period from 0 to 8.5 days p.c., and included almost 3600 embryos exposed to 50 R in two equal fractions separated by different times. Exencephaly was the malformation particularly studied. When the dose fractions were separated by less than 4 hours, there was no difference compared with the effect of a single combined total exposure. Longer fractionation intervals seemed to reduce the incidence of anomalies but not the incidence of early intra-uterine death.

304. Russell, Badgett and Saylors (298) carried out rather more elaborate experiments, studying different effects (mortality, weight loss, long-term survival, fertility and morphology). They tested the effects of *(a)* 171 R (0.096 R/min) accumulated during the first 2 weeks of pre-natal development; *(b)* 200 R given acutely (83 R/min) at one of seven stages of pregnancy (0.5, 1.5, 4.5, 7.5, 9.5, 11.5, 13.5 days p.c.); *(c)* 191 R administered in 15 daily acute fractions of 12.8 R within the same time. As expected (see chapters II and III), acute irradiation produces a great variety of incidence and severity of effects, strikingly dependent for their type and degree on the stage at irradiation. Chronic irradiation, on the other hand, induced no special effect, except for shortening of the fertility period in females. Daily acute fractions had no more adverse effects than continuous exposure.

305. On the likely hypothesis that cell killing might be the main cellular effect responsible for the initiation of malformations (see paragraphs 280-283), these results were interpreted to mean that cell killing in embryos is lower with chronic than with acute irradiation. The fact that single doses of radiation given at the sensitive periods were effective in causing abnormalities, while lower doses within the same sensitive periods were not, is compatible with the model reviewed in paragraphs 284-287, assuming that the total dose administered during these periods was insufficient to inactivate more than the threshold number of cells required for the production of abnormalities. The difference to Auerbach's findings (7) could be explained by the assumption that cyclical periods of higher sensitivity could have been responsible for the potentiation of the effects by fractionation.

306. More recently Yoshizawa and Ueda (373) submitted six different groups of ddY mice at 11 days p.c. to 200 R given at the rate 1-100 R/min. The body weight and the delay in ossification of the caudal vertebrae scored at 18 days of pregnancy were assumed as indicators of whole-body and systemic damage. Both these parameters were found to be in good relationship to increasing exposure, but they were insensitive to the exposure rate within the range indicated.

307. The work of Kriegel and Langendorff (159) does not allow a direct comparison of the effects of acute and fractionated doses. It gives, however, information on the incidence of malformations and of lethal effects induced by fractionated, daily x-ray exposures administered during the total pregnancy period of the mouse. Levels of 2.5-10 R per day had an effect on embryological development. At 20 R per day a small increase in resorption rate and in malformation incidence was observed together with a decreased weight of the conceptus. Fractionation régimes of 80 R per day caused the interruption of pregnancy. Another series with 80,

120, and 160 R per day for limited pregnancy periods of days 11-16, 11-13 and 14-16 p.c. was reported by Kriegel and Reinhardt (161). At 18 days of gestation striking effects on growth and malformations of the tail and the extremities were obtained.

308. Konermann (152, 153, 154, 155) published a series of papers dealing specifically with the effects of fractionated and chronic irradiation. In the first report (152), the differential stage sensitivity was particularly examined at exposure regimens of 5-100 R per day. It was shown that the developmental stage, rather than the dose, affected the type and severity of the morphological damage. Studying the effects on foetal weight and the teratogenic effects as a function of dose and time, the paper concluded that limited processes of restitution may correct in part the primary radiation-induced lesions. The second paper (153) dealt with skeletal malformations and their relations to external morphological abnormalities.

309. The third contribution (154) is of particular importance for comparing the effects of fractionated and continuous irradiation during blastogenesis, organogenesis and foetogenesis, and also during the whole period of gestation. Resorptions, intrauterine death, malformations and foetal weight were the end-points studied. The degree of effect for a given fractionated dose was compared with the effect of the same dose under continuous irradiation. The minimum dose, the 50-per-cent dose and the maximum effective dose for lethal and teratogenic actions were compared, and it was concluded that continuous exposure required 1.5 times higher doses than fractionated exposure for the same degree of effect. This factor was considered to be small compared to the ratios between the dose rates of the two exposure types ($2\ 10^3$ to $2\ 10^4$), thus confirming that the stage sensitivity of the embryo is by far the most important factor determining the final outcome of irradiation.

310. The last paper (155) examined the recovery mechanisms in the embryo by the use of the exposure-fractionation technique. Groups of mice irradiated with daily fractions (10-80 R per day) on day 1-5 p.c. or 6-13 p.c. were compared with other groups receiving a second series of doses on day 14-18 p.c. The foetuses were examined on day 18 p.c. The percentage of normal, abnormal and dead animals and their weight showed that sensitivity during the foetal period was not enhanced in the dose range studied by previous irradiation during blastogenesis. On the other hand, most parameters studied, and particularly weight loss, showed an effect of potentiation, by pre-irradiation during organogenesis.

311. Observations on mice irradiated continuously throughout gestation were also reported by Warren and Gates (358). A recent paper by Friedberg *et al.* (91) dealt with the survival of mice irradiated in the pronuclear-zygote stage with single or fractionated doses of x rays (60 rad or 30 rad + 30 rad) and fission neutrons (14 rad or 7 rad + 7 rad). The interval between the two doses was 4 hours. No significant differences were observed between the single-dose and the split-dose groups. In view of the changes in sensitivity during the fractionation interval (see also reference 299), the effect of the split doses could be interpreted assuming that the first fraction blocks the embryo from proceeding towards a less sensitive stage.

312. There are also experiments on the effects of dose rate and fractionation in other animal species. In rats, Sikov and Lofstrom (315), using the experimental schedule described in paragraphs 297-300, produced evidence of a reduction of the teratogenic action by dose protraction. Brown *et al.* (45) bred female rats under gamma radiation levels of 2-10 R per day. They found no essential difference in the litter size and in the number of offspring born in six successive litters of the mothers living in the continuous radiation field. However, at 20 R per day, the fifth litter was drastically reduced in size and the sixth litter had no offspring born. Malformations were not seen, but there was evidence of some weight reduction. Animals born from the third litter were tested for fertility and found to be normal up to 5 R per day but sterile at 10 or 20 R per day.

313. In a series of papers on morphological damage of brain structures induced by fractionated irradiation, Brizzee *et al.* (42, 134) reported the results of exposures of 13.5 and 14-day embryos singly to 150 R or to various fractionation patterns within the same half-day period. The damage to brain structures shown at 19.5 days p.c. was higher after single exposures and decreased progressively when two 75-R fractions were administered at intervals of 1, 3, 6, 9 or 12 hours. Subdivision of the dose into 2, 3, 5 or 9 equal fractions over the same time also progressively decreased the effect, which was less in these animals than in those of the previously mentioned variable-interval group. It was concluded that, for a fixed dose over a given time, the size of individual fractions is more important than the number of fractions in determining the final effect.

314. In another experiment (41) a group of embryo rats was irradiated on day 13 p.c. with single whole-body exposures of 12.5-200 R. A second group received 100 R on day 13 p.c. and, after 9 hours, a second exposure varying of 12.5-125 R. The damage to the olfactory lobes and the cerebral hemispheres, as seen on day 19 p.c., was small for the exposures between 12.5 and 87.5 R in the single irradiation groups and for exposures between 12.5 and 50 R in the split-irradiation groups. At higher exposures, the damage manifestly increased in both groups.

315. The logarithm of the mean depth of the cortical zone was plotted *versus* the radiation exposure. The plot showed a shoulder up to 62.5 R, followed by an exponential decrease to 200 R. Some shoulder (up to 50 R) was also evident in the split-dose series, which could indicate the presence of some degree of cell recovery with time between the two doses. Treatment of the animals with hypothermia to 20°C during the fractionation interval (40, 43) reversed to some extent the decreased effect of the split dose, indicating a possible inhibition of the repair processes.

316. In the experiments of Martin (184), an exposure to 160 R on day 18 p.c., at rates of 1, 3 or 47 R/min produced some effect on the pre-natal and post-natal

body weight. The amount per cell of protein and RNA in the brain were not affected consistently by the irradiation. The cerebral cortex of the irradiated animals, however, weighed less than that of controls and had a lower content of DNR, RNA and proteins, and this effect was apparently not influenced by the exposure rate. The other portions of the brain showed partial recovery of the total DNA, compared to the control value. Exposure rate had some effect, since recovery was higher in the 1-R/min group and progressively lower at higher exposure rates. After 2 months of life, the brain of the controls and of the 1-R/min group contained more DNA than those of the groups irradiated at higher exposure rates.

317. Reviewing the information on the influence of exposure rate, Brent (30) concluded that exposure rates below 5 R per day did not produce observable deleterious effects in the surviving offspring. He also reported results of experiments in which rat embryos were exposed to 150 R on day 9.5 p.c. at different rates from 0.5 to 100 R/min. These results indicated that protraction reduced the resorption and the growth retarding effects. Higher exposure rate groups had a higher incidence of malformations and were more severely malformed. Malformations like anencephaly, microcephaly, microstomia, evisceration, renal agenesis and absent pinna did not appear in animals irradiated at low rates. However, any malformation with an incidence higher than 45 per cent in the high exposure-rate groups was also seen following protracted irradiation.

318. After obtaining an exposure-effect curve (50-200 R gamma radiation) for ocular malformations in rats at 11 days p.c. to establish a suitable level for exposure-rate studies, Strange and Murphree (338) irradiated these embryos with 100 R given at 1, 2, 3.3, 5, 10, 25 and 47 R/min. Grading the ocular damage observed at 30 days of age, they showed an increase of eye abnormalities up to 3.3 R/min, a gradual but not significant further rise up to 10 R/min and a significant drop at 25 and 47 R/min below the levels observed at 3.3 and 10 R/min. The increase in susceptibility of this system with increasing rate of exposure falls within the usual pattern found in dose-rate experiments and is compatible with the commonly accepted notion that any end-effect results from competing phenomena of damage and repair, both of which are time-dependent. However, the fall at the high exposure rates is a surprising finding. The authors suggest that there may be a limited and short period of sensitivity in the life of the target cells and that the probability of hitting this sensitive stage with an effective dose might decrease at very high exposure rates. Whatever the explanation may be, these data represent further evidence that the complexities in the division and differentiation mechanisms of a developing embryo, by comparison with a more conventional cell population at equilibrium, may justify unusual findings with respect to specific end-points.

319. Continuous low-intensity gamma irradiation was given to pregnant rats at 50 R per 20-hour day, starting on the first day of pregnancy (67). Embryos, foetuses and uteri were examined at 10-20 days p.c. for growth and the presence of abnormalities. At this exposure level the total number of implants was not affected but an increase of mortality was seen at day 12 p.c., the great majority of lethal effects occurring before day 15 p.c. The pre-natal growth of the embryos was greatly delayed and many types of abnormalities, including microcephaly, anophthalmia and skeletal malformations, were also found.

320. Malformative defects of the limb possibly associated with a specific dose rate have been described in irradiated cattle, sheep and swine by Erickson and Murphree (82) and McFee *et al.* (188). Murphree and Graves (211) studied the effect of exposure and exposure rate on lambs irradiated on day 23 p.c. with ^{60}Co gamma rays. Pregnant ewes were exposed to 282 R at a rate of either 1 or 47 R/min. About 15 per cent of the ewes exposed to 1 R/min and 38 per cent of those exposed to 47 R/min died about 23 days after irradiation. The foetuses recovered from these dead animals were all smaller than normal. In the 1-R/min group, 4 ewes produced 4 deformed lambs; in the 47-R/min group, 1 ewe had twins, one of which was deformed. Of the 5 deformed lambs, 3 had ulnar aplasia and 2 aplasia of the ulnae and femora. A second series with a total exposure of 188 R was performed. Out of 28 lambs born in the 1-R/min group, 10 had deformities of the extremities, eyes and head. In contrast, only 1 out of the 29 lambs born in the 47-R/min had leg deformities.

321. In conclusion, the majority of the data available for most species indicate a decrease of the cellular and malformative effects by lowering the dose rate or by fractionating the dose. However, examples of deviations from this trend have been well documented in a few instances and are not inconsistent with the knowledge about mechanisms of the teratogenic effects. It is therefore impossible to assume that dose rate and fractionation factor have the same influence on all teratological effects.

3. The effect of oxygen

322. There has been some research on the influence of oxygen pressure on the induction by radiation of teratogenic effects in mammalian embryos. Russell *et al.* (305) (also reported in Russell (295)) showed that hypoxia resulted in substantial protection in mouse embryos exposed to 100-400 R on day 11.5 p.c. Hypoxia was induced by breathing a mixture of 5 per cent oxygen and 95 per cent helium. A number of end-points were scored at birth, such as the mean birth weight, mortality, tail length and shape and foot malformations. Irrespective of the shape of the dose-effect curves for the indicators examined, the magnitude of the protection afforded by the low oxygen pressure was approximately the same for all indicators. Oxygen seemed to act as a simple dose-modifying agent, with an oxygen-enhancement ratio of 2-3.

323. Rugh and Grupp (280) treated pregnant females (8.5 days p.c.) with a mixture of 6 per cent oxygen and 94 per cent nitrogen for 8-13 min, and then exposed these animals while still unconscious to 200 R. Non-irradiated animals kept at low oxygen pressure

served as controls. The foetuses were observed by uterine dissection on day 18.5 p.c. Anoxia alone had no effect on the embryos. Low oxygen pressure in combination with radiation resulted in 71 per cent normal embryos, while the same dose at normal oxygen pressure would yield only 41 per cent normal embryos. Resorbed embryos and exencephaly in foetuses, after irradiation in hypoxia, was recorded as 16 and 13 per cent against 34 and 21 per cent in the corresponding controls. Therefore anoxia produced some degree of protection against both lethal and teratogenic damage. Since there are insufficient data to draw a dose-effect curve, the numerical factor to be attached to this protective action cannot be evaluated with precision.

324. Uterine vascular clamping (35) is an alternative procedure for producing anoxia of the embryo. When this technique was applied to one uterine horn of the rat for 45 min on day 8 or 9 p.c., it had minimal detrimental effect on foetal growth and survival. This technique has been shown to protect against radiation-induced lethality and weight loss (36). Another method of producing hypoxia indirectly, by inducing hypoglycemia with insulin, was found to be totally ineffective in the mouse (280).

325. It may be concluded that the oxygen effect in the embryo is similar to that observed in the irradiation of other cellular systems of the adult animal.

4. Radioprotective and radiosensitizing drugs

326. There are numerous reports on the effect of radioprotective drugs upon the induction of embryonic and foetal damage by radiation. The protective effect of cysteinamine (269) and cysteamine (369) had been shown in irradiated foetal mice with respect to survival, growth rate, weight and induction of malformation. Rugh and Grupp (280) examined the effect of these substances on CF1 embryos exposed to 200 R on day 8.5 p.c. When 3 mg of cysteinamine were administered IP 30 min prior to exposure, the incidence of normal foetuses increased from the expected 41 per cent to 73 per cent, resorption decreased from 34 per cent to 8 per cent and exencephaly incidence decreased from 21 per cent to 16 per cent. In the case of cysteamine (3 mg IP withing 30 min of exposure) the shifts were: normal animals, from 41 per cent to 80 per cent; resorptions, from 34 per cent to 12 per cent; and exencephaly, from 21 per cent to 8 per cent. These two drugs therefore appeared to act beneficially, increasing survival and reducing the incidence of malformations in the irradiated embryo. However, these experiments with a single dose of radiation do not allow the derivation of good estimates of the protection factor. AET did not counteract the radiation effects.

327. Numerous other treatments (hypotonic and hypertonic solutions, dextrose, alcohol, chlorpromazine, and homogenates of spleen, marrow and liver) tested for possible action in the same experiments (280) were also found ineffective. Konermann (156) reported complete dose-exposure curves, in the range 150-450 R delivered in single irradiations at 3, 6, 9, 12 and 15 days p.c., with and without cysteinamine (a single amount of 200 mg/kg given 8 min prior to irradiation). The end-points, checked by uterine dissection on day 19 p.c., were morphologically normal and abnormal animals, malformed and dead foetuses, frequency of embryonic deaths, foetal and placental weight, and skeletal malformations. Marked differences were observed in the relative protective effect of the drug for various radiation doses and various stages irradiated. Protection was shown to exist both in the pre-implantation and in the organogenesis period, and the value of the protection factor was different for different malformations.

328. In the rat, Starkie (331) reported a partial inhibition by cysteamine of the radiation effects on the testis. Animals were irradiated at 17 and 21 days p.c. with doses of 50-150 rad and the testes examined histologically at the age of 25 days. Kalinina (140) tested the effects of a number of possible radioprotective substances, such as phenatine, magnesium sulphate, chlorpromazine and mercaptamine, injected in appropriate doses 15-20 min before irradiation (200-300 R on day 11 p.c.). Embryo and placental weight, mortality and malformations were scored on day 22 p.c. The mortality rate, and to some extent the incidence of malformations, were lower in the injected groups.

329. Baev *et al.* (9) tested the protective effect of cysteinamine and cysteamine in the case of fractional exposure to radiation throughout gestation (40 R per day for 20 days). The irradiated offspring were followed to adulthood, and the changes in litter size, post-natal death and body weight were noted. While protection was afforded by both drugs in regard to litter size and weight, the post-natal death rate was unchanged. Twenty females and 24 male offspring surviving after drug treatment and tested when sexually mature (10) showed greatly reduced reproductive performance, sterility and increased frequency of dominant lethal mutations. A protective effect of cysteinamine, most pronounced after completion of organogenesis, was also described by Kirushenkov (151). Treatments of pregnant females with proteolytic enzymes (348) or with progesterone (353) were also reported to act beneficially in protecting the foetus from radiation-induced malformations. In contrast to that result, small doses of iodacetamide (194) and miracil D (193) were shown to act synergistically in potentiating the teratogenic action of radiation.

330. It may be concluded that many of the known radioprotective drugs which are active in modifying the radiation response of adult systems are also effective against the damage caused by irradiation *in utero*. In the latter case there is evidence that the protection factor may vary according to the stage irradiated and to the particular type of damage analysed (lethality, growth disturbance and type of malformation).

F. SIGNIFICANCE OF THE EXPERIMENTAL DATA TO MAN

331. The majority of authors who have contributed experimental information on teratogenic effects of radiation have considered, directly or implicitly, the

applicability of their findings to the human species. A number of papers have also specifically dealt with this subject (see for example 295, 297, 301, 304, 261, 265, 268, 27, 136, and 345). The following paragraphs discuss the opinions expressed by the authors and summarize the views of the Committee regarding present knowledge and the need for future research in radiation teratogenesis.

332. It is clear from all the preceding sections that for teratogenesis, more than for any other early or late somatic effects of radiation, data on humans are extremely limited. Apart from growth disturbances, microcephaly, mental retardation and a few other defects of less importance, there are very few systematic data applicable to man, particularly at the low doses of interest. However, the scanty data available for other abnormalities are probably sufficient to permit the assumption that the effects observed in animal experiments also occur in man. The animal experience therefore appears to be of unique and special importance for the assessment, even if only qualitative, of malformative effects in man.

333. Animal research has established two important qualitative facts: *(a)* there is a uniform pattern in the sequence and timing of the early developmental stages in the mammalian species tested (see chapters I-III); and *(b)* there is a general similarity among the various species with regard to the type of malformations induced and the teratogenic mechanisms (see chapters III-VI). However, these facts are in themselves insufficient to justify any direct quantitative extrapolation of effects between species for a number of reasons: *(a)* the great specificity of the malformations induced at comparable stages in different species and even among different strains of the same species (see chapter II); *(b)* the species difference in the duration of the foetal period (see table 3), which causes marked discrepancies in the final state of the malformations and causes serious obstacles in comparative studies between animals and man as, for example, with the central nervous system (141); *(c)* the extremely variable form of the dose-effect relationships in different species (see chapter III).

334. For all the above reasons there is general consensus (304, 136, 267, 268, 344 and 345) that data applicable to man can only be derived from human epidemiological studies. These studies are, however, not available at present, at least on the scale required and at the low doses of interest. The Committee believes that this point should be particularly emphasized so as to discourage numerical extrapolations not sufficiently justified by present knowledge.

VII. SUMMARY AND CONCLUSIONS

A. GENERAL

335. Scientific contributions concerning the somatic effects on experimental animals irradiated *in utero* have been reviewed with the aim of extracting information relevant to the assessment of similar effects in man. Data on embryological effects in the human have also been reconsidered and updated. In addition to lethal effects, effects on growth and development, and miscellaneous pathological consequences of the exposure, malformations have been particularly examined as typical sequelae of irradiation *in utero*. The amount of data directly or indirectly related to this subject is indeed rather large, but most of it has been produced with the ultimate object of analysing developmental mechanisms. Thus, the quantitative information to be gained on the main radiobiological variables (dose, dose rate, fractionation, LET etc.) is definitely insufficient, particularly in regard to the human embryo. The Committee emphasizes, however, that appropriate evaluation of whatever data is available may help to set the scanty knowledge on man into a better perspective. It may also strengthen the qualitative extrapolation of effects between species, pending the quantitative assessment of radiological hazards which must of necessity be carried out in man.

B. METHODOLOGY AND TECHNIQUES

336. Among the variables or conditions affecting the induction of embryological effects in mammals, the genetic constitution, the breeding characteristics of the species and the reproductive history of the mother have been shown to influence the degree of the lethal and teratogenic responses of the conceptus; other variables have a relatively minor but not trivial importance. There are well defined periods of maximum sensitivity during gestation for the induction of different types of malformations; these periods are related to the stage of major differentiation of the relevant embryonic structures. It is possible to reconstruct developmental tables in animals and man to compare the approximate critical times for the occurrence of certain recognizable ontogenic events and to facilitate rough intercomparisons of pregnancy stage and of embryonic differentiation. In animals, a number of variables affecting the scoring of lethal, developmental and malformative effects have been recognized, and their importance for correct radiobiological assessments has been discussed. Regarding the human experience, insufficient knowledge of the conception time, lack of precise dose estimates, paucity of irradiated groups and difficulties in the selection of control groups prevent any precise estimate of hazard. Following an analysis of the main periods of pre-natal development in various mammals, a systematic review of embryological effects is provided.

C. THE PRE-IMPLANTATION PERIOD

337. Killing of the embryo prior to implantation stands out during this period as the most conspicuous effect of irradiation, which may be expressed in polytocous animals as death of single embryos or death of the entire litter. Post-implantation and post-natal death are regarded as relatively less important hazards of the exposure during the pre-implantation stages. In addition to considerable differences in sensitivity between animal species, there have been described substantial changes in the susceptibility to this type of damage within each species, particularly during the early segmentation of the fertilized egg. In the mouse, the species where the most

data have been obtained, a number of reports point out, in good agreement, that the absolute increment of embryonic loss soon after fertilization could be of the order of 10^{-2} R^{-1}. Chromosomal damage to the irradiated blastomeres followed by degeneration of the primitive embryo cells appears to be the major mechanism responsible for embryonic death. During this stage, the reduction of body growth is not an effect clearly and universally recognized. The malformations documented, particularly in the nervous system of the rodent, may also be regarded as less frequent events, in comparison with their higher frequency during the following stage of organogenesis.

D. THE PERIOD OF MAJOR ORGANOGENESIS

338. Lethal effects during this period may be studied through several indicators, such as the complete failure of pregnancy, the decrease of the average litter size, the mortality *in utero* of the irradiated embryo and the neo-natal and long-term survival of the live-born animals. Concerning pre-natal mortality, the sensitivity of the embryo is highest soon after implantation and during early organogenesis, with a subsequent decrease at later times. The shape of the exposure-lethality function appears to change substantially with the developmental stage, and the majority of data point to curvilinear functions. There is some information on the effective doses, particularly in rodent species, in regard to pre- and neo-natal death. They have shown LD_{50} values lower than 150 R at the pre-implantation and early organogenesis stages, after which these values invariably increase to attain during the foetal stages levels comparable to the post-natal LD_{50}. Long-term reduction of life span may also be documented in rodents for exposures of a few hundred roentgens; these effects strongly depend on a number of genetic factors. Information in man is virtually absent.

339. Data obtained from several animal species indicate that the period of major organogenesis is very sensitive to growth disturbances, probably more than the following foetal period but definitely more than the preceding pre-implantation phase. When growth defects are scored prior to birth, the magnitude of the growth deficit appears to vary with the interval between irradiation and observation. Only in very few cases are there sufficient data to evaluate the net effect of the dose after a fixed post-irradiation time or at the time of maximum effect. Growth disturbances induced by irradiation of the embryo but persisting in adult life and expressed at the whole-body or organ level have also been described in the experimental animal.

340. In spite of the great wealth of information on malformations induced during organogenesis in various animals, it appears extremely difficult to summarize these data into any coherent quantitative conclusion for the following reasons: the species and strain variability; the scarcity of complete dose-effect series; the different criteria of scoring the induced aberrations; and the different embryological and physical conditions under which irradiation has been performed. A few general statements may however be justified:

(a) With due regard to developmental and anatomical differences between species, similar classes and types of malformations occur upon irradiation at comparable developmental ages. This finding is sufficiently general to suggest that man might not represent an exception in this respect, even though no quantitative comparisons between experimental animals and human species would be warranted at present;

(b) Within each species there is a well defined time at which each malformation may be induced. Increasing the dose usually results in a spread of the sensitivity peak and in an increase of the malformation incidence. In some cases, biphasic periods of sensitivity are observed, related presumably to different mechanisms of induction resulting in similar malformative end-points;

(c) The time specificity of each malformation usually coincides with the major phase of differentiation and organization of the relevant structure. Cell killing followed by an arrest of organ development at some rudimentary stage may be identified as the mechanism initially responsible for the malformative event;

(d) Data are too few and their variability too high to allow any firm conclusion about the relative radiation susceptibility of different embryological structures within each species and, even less, between various mammals, but malformative responses have in a few cases been described down to exposures of 5 R;

(e) In the vast majority of cases the malformation incidence has a curvilinear trend with dose, implying relatively less effect per unit dose at low than at high doses. However, linear or quasi-linear dose-effect relationships are not uncommon, particularly when embryologically or topographically related malformations are grouped together in such a way to express the malforming effects as the ratio of malformed to normal animals, irrespective of which type or number of malformations might affect the malformed conceptuses. Such cases have been documented only in animals; it is conceivable that in the case of animals absolute increases in the incidence of malformed foetuses of the order of 5 10^{-3} R^{-1} of low-LET radiation delivered at high dose rates might occur. It should however be emphasized that such estimates are very tentative and their projection to other species and particularly to man is unwarranted;

(f) In man the best documented type of malformation is microcephaly, which in its most extreme cases is accompanied by mental retardation. Following high acute doses its incidence has been tentatively estimated at around 10^{-3} rad^{-1}, with the lowest effective doses ranging between 10 and 150 rads under different conditions of exposure;

(g) Other epidemiological surveys in man following doses in the region of 1-20 rad have given either negative or non-significant answers; they could only be of some value in excluding the possibility that at these doses the human embryo could be 10 times more sensitive than the incidence of malformations at higher doses would imply.

341. Among other miscellaneous effects of irradiation during embryogenesis, changes in fertility and fecundity, cataract, and relatively minor defects have also been studied in animals.

E. THE FOETAL PERIOD

342. Lethal effects have been described in the rodent for irradiation during this period. Although limited to selected ages, these data show consistently a reduction of sensitivity with advancement of foetal age. There is also some information in man obtained in cases of radiation-induced therapeutic abortion and, with some dosimetric uncertainties, following the A-bomb explosions in Japan. This information refers to both pre- and post-natal radiation lethality and to long-term effects on survival.

343. Generalized and local growth disturbances are commonly described effects of irradiation of the foetus, and they are seen in many cases to persist throughout the extra-uterine life. In man numerous studies agree in pointing out a growth-retarding effect of irradiation manifested by a reduction of various body parameters, in addition to microcephaly and mental retardation.

344. It is commonly accepted that as foetogenesis proceeds the malformative effects of radiation become more difficult to document macroscopically since, for the same radiation dose, the size of the anatomical structures involved becomes progressively smaller and the resulting functional defects less important. It should however be expected that these subtle malformations, rather than the major defects induced at organogenesis, would be the ones more likely to survive and to result in the main social burden. Data in animals relate particularly to effects on the eye, the nervous system and the gonads. In man, besides microcephaly, heterochromia of the iris has also been described.

345. Other pathological effects on the foetus regard the haemopoietic system at the bone-marrow and peripheral level; haemopoietic failure at this stage is closely related to the whole-body lethal effect. Laboratory investigations carried out on samples of children and adolescents irradiated *in utero* have shown a good capacity for recovery of whatever haematological damage might have been induced soon after exposure. The question of a possible alteration of the sex ratio of children born to parents exposed *in utero* is still controversial.

F. INTERNAL IRRADIATION

346. The interpretation and evaluation of the developmental effects of irradiation *in utero* by incorporated radionuclides must be based on a precise knowledge of doses received at specified dose rates by the mother and the conceptus at any given developmental stage and in any sensitive structure under various conditions of treatment. The information available falls far short of this objective. The effects observed are similar to those described for external irradiation; they are, however, less specific owing to the fact that exposure to the nuclides usually extends throughout embryonic and foetal stages. Among the main variables known to interact in the production of these effects, the following have been identified: the physical nature of the nuclide and the chemical form in which it is administered, the route and schedule of administration, the dose, the developmental age at irradiation, the animal species, and the possible role of the irradiated maternal organism.

G. MECHANISMS OF RADIATION TERATOGENESIS

347. Most of the data support the contention that indirect effects of the mother's irradiation do not play a major role in the induction of malformations, particularly at the low doses of interest for radiation protection. On the other hand, it is conceivable that high doses given to the mother might also influence the mortality of the embryo and foetus.

348. Malformations are typical sequelae of damage inflicted by radiation on a developing organism. They are produced through a disturbance or disruption of the orderly sequence of ontogenic events that result in a developed foetus. At the subcellular level it appears quite unlikely, even in the absence of much direct information, that the primary biophysical events taking place in embryonic tissues may grossly differ from those occurring in adult tissues. At the cellular level chromosomal aberrations are thought to be responsible for the loss of reproductive capacity of the irradiated embryonic tissues. This notion is in agreement with what is observed in adult tissues and has been substantiated for many embryological structures as well. Selectivity of the damage with respect to different tissues which are seen to respond at different times is postulated to depend on changes in the intrinsic radiosensitivity of cells, on the variable kinetic parameters of the different embryonal tissues and on the capacity for repair and repopulation of each tissue. Radiation damage to regulatory mechanisms could also be regarded as a component of the teratological damage, but up to the present all these processes have only been considered theoretically and have never been experimentally documented. Simple mathematical models for the development of malformative damage have also been proposed and they are not incompatible with the observed dose-effect relationships. The analysis of such relationships in order to gain a better knowledge of mechanisms would however be unwarranted in the absence of further experimental evidence.

349. Regarding the possible existence of thresholds in dose-effect relationships there is consistent evidence showing that doses as low as 5 rad may still be effective in inducing selected malformations. Direct experimental tests of the absence of thresholds in this dose region would tend to exclude their existence at even lower doses. Theoretically, the possibility does exist that thresholds might occur at even lower doses, but experiments of sufficient precision to reveal them would be technically difficult or even impossible for statistical reasons.

350. Data on the effect of radiation quality and energy in respect to teratogenic damage are extremely rare and definitely insufficient for a more than speculative knowledge of the relevant RBE factors. Within these limitations it appears, however, that present information is not incompatible with the notion that higher RBE

factors might apply to the more densely ionizing radiations and to the case of dose protraction. More refined information on RBE factors relating to different malformations is absent.

351. Data in many species would indicate some decrease of the malformative effects induced at low dose rates or with dose fractionation. However, the nature of the embryonic material is such that under special conditions a potentiation, rather than a reduction, of effect might be possible; a few examples of such effects have indeed been found. In any case, it seems impossible to assume that the same reduction factors for low dose rate or fractionation might apply to all effects induced by radiation in embryonic tissues.

352. Anoxia and treatment with radiosensitizing and radioprotective drugs have been shown consistently to act as modifiers of the damage to the embryo in the same sense and by about the same factors as for adult tissues. The protection afforded is variable and depends on the age of the embryo and the type of damage observed.

H. RESEARCH NEEDS

353. The qualitative value of animal experimentation for the assessment of human effects in no way limits its usefulness, particularly in view of the lack of precise information for many types of teratogenic effects in man. The Committee has identified several fields where further information would be highly desirable. The fields, which relate to both human observations and animal experimentation, are as follows:

(a) Studies of stochastic[2] *versus* non-stochastic effects occurring in the embryo and foetus at various stages of development would appear to be of great value for their implications on risk assessments in man. These studies would imply the ascertainment of some cellular parameters which have been postulated in some models (see paragraphs 284-287) but have never been determined experimentally;

(b) Too little is known about whether, and to what degree, the mechanisms and kinetics of inactivation, recovery and repair of adult somatic cells and tissues apply also to embryonic and foetal systems (see chapter VI, section B). A better understanding of the primary effects might also be of help in developing more refined models of teratogenic damage, reflecting the effects of the main radiobiological variables (dose, dose rate, LET, fractionation etc.). For example, the concepts of microdosimetry (146) have so far not been extended to damage *in utero*, and such an approach in this field (see chapter VI, section C) might lead to a re-evaluation of old data or to the design of new and more precise experiments;

(c) The study of pathogenetic mechanisms and development patterns of malformations are thought by some (267) to be of little value at present, while actions in the fields of etiology and prevention are advocated as the future major steps. This opinion could be supported in regard to accumulating further descriptive work on malformations. However, the Committee believes that extensive data on specific malformations as a function of dose (and particularly at low doses) and in several animal species would be highly desirable and would considerably increase our confidence in interpreting the estimates in man (136);

(d) In planning and performing such experiments, the co-operation of radiobiologists and embryologists is essential to ensure that such large and costly undertakings would eventually be of value for human assessments. In addition to the technical points underlined in chapter I, the selection of relevant end-points for experimentation should reflect the fact that relatively minor morphological and functional defects in man may be more important than extensive malformations which would in most cases be incompatible with extra-uterine life;

(e) One area where data are extremely scarce is the influence of radiation quality on malformation induction (see chapter VI, section D.1). More systematic experiments, especially at low neutron doses, should be performed with various LET spectra and at different stages of embryonic development to accumulate more precise data on the RBE values for different types of malformations, which at present are only largely estimated on the bases of inferential evidence (256);

(f) Effects with dose fractionation and low dose rate also deserve better attention, in view of the possibility that special régimes of fractionation or particular dose rates may be more harmful than single acute doses (see chapter VI, section D.2);

(g) Internal irradiation by various nuclides has only been episodically explored. It will be necessary to extend present experience to the potentially most hazardous and important nuclides with studies of uptake, distribution, clearance, dosimetry and effects on both the mother and the foetus;

(h) There should be more surveys to evaluate the type and frequency of x-ray examinations of the lower abdomen of pregnant women and the resulting doses to embryos (see Annex I). Such data have already been obtained in some cases (44, 359, 361), but they should be extended to different socio-economic and medical situations in order to obtain representative estimates of the risks involved;

(i) Finally, no occasion should be lost for accumulating data on irradiated human populations; only on the basis of such data are refined risk estimates possible. It is essential, therefore, to continue the study of the irradiated populations already surveyed. Epidemiological surveys on other population groups yet to be identified will also be required to improve the present incomplete knowledge, at least to the point of excluding the possibility that exposures might be more hazardous to man than to experimental animals.

[2] In the present context the terms "stochastic" and "non-stochastic" are used as defined for the purpose in question by the International Commission on Radiological Protection: "stochastic effects are those for which the probability of an effect occurring, rather than its severity, is regarded as a function of dose, without threshold. 'Non-stochastic' effects are those for which the severity of the effect varies with the dose, and for which a threshold may therefore occur" (129a).

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Printed in Austria
id.77-3247–October 1977–5,000

Price: SU.S. 28.00
(or equivalent in other currencies)

United Nations publication
Sales No.: E.77.IX.1