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 Grieben 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 uranium-ore deposits, uraniumiferous 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 CP1 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 are 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 \textit{in utero} is discussed in Annex I.

179. A significant decrease of the \textit{in vivo} and \textit{in vitro} incorporation of labelled amino acids 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 \textit{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 polyosomes 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).

\section*{IV. THE FOETAL PERIOD}

\subsection*{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 Wohlf fromm (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 \textit{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, severely 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 \textit{in utero} mortality. Rugh and Wohlf fromm (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 G Owen (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 S i k o v 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. Murphee 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 LD50/30 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 LD50/30 for the adults in both species, the LD50/30 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 LD50. 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 LD50/30 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 Harris (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 (~ 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 lzadian (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 ex-
posures 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, Koner-
mann 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 spe-
cifically 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 by
induction 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
wrists 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 circum-
ference, 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 exami-
nations 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
tibia, inguinal hernia, microcephaly etc. Both the
radiation dose estimated by the number of radiographs
and the time at irradiation could not be clearly
related to the incidence and type of malfor-
mations. 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 malfor-
mations, because of the relatively small sample size.

C. MALFORMATIONS

1. General

209. It is widely accepted that following the termina-
tion 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 differenti-
tated 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 malfor-
mations 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 Mellengaard (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 haemopoesis shortly after irradiation. Among the more recent contributions, Reinecke (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 d4Y 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 Wohlfrom (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 age was 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 phenomenon 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 interspecies 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, Wohlfmark 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 60Co 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 beyond 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 leucocyte, 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 leucocytes 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.
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.

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.

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

The first reported observations on the effect of injected radioisotopes in the foetal mammal are those of Begg (11). He injected radon solutions in amounts of tens of microliters 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.

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

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. Isotoma 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. LD50 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).

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 60Co 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 60Co 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 LD90 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 60Co gamma rays was convex upward, indicating a decreased killing effectiveness of the gamma-ray treatment at low doses. By comparing individual gammaray 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, leucoopenia, 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 (3HTdR) 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 3HTdR 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 3HTdR 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 3HTdR (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 32P 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 32P was given early in pregnancy.

253. Sikov and co-workers carried out a series of systematic quantitative studies on the consequences of 32P 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$_{10}$, for lethality of the foetuses before day 14 p.c. These LD$_{10}$ 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$_{10}$ 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 equaled 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 $^{90}$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 newborn 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$_{30}$ 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 Henicron (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). Ilijin 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 foeto-placental 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 LD₃₀/₃₀ of ²⁴¹Am 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 ²⁴¹Am varied from 6:1 to 2:1 (201)), the LD₃₀ 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 to present 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 compund 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 organotopic 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, Chung 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 repressive 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 aberrations (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

<p>| TABLE 5. SYNOPSIS OF THE LOWEST TERATOGENIC DOSES OBSERVED IN MICE AND RATS |
|---------------------------------|------------------|--------------------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Gestational age (days p.c.)</th>
<th>Exposure (R)</th>
<th>Effects observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.5</td>
<td>5</td>
<td>Increase in resorption frequency</td>
<td>278</td>
</tr>
<tr>
<td></td>
<td>0.5-1.5</td>
<td>15-20</td>
<td>Exencephaly</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td>0.5-1.5</td>
<td>5</td>
<td>Polydactyly</td>
<td>277</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>5</td>
<td>Increase in resorption frequency</td>
<td>231</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>5</td>
<td>Skeletal malformations</td>
<td>297</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>5</td>
<td>Decrease in litter weight (winter sample)</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>5</td>
<td>Hydramnios</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>5</td>
<td>Reduced tail length</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5-8.5</td>
<td>25</td>
<td>Malformations of the axial skeleton</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>25</td>
<td>Hydroceplalus, flexion of the spinal cord, architectural changes of ependymal cells</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>50</td>
<td>Eye defects</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>0-8</td>
<td>5-25</td>
<td>Growth disturbances</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>12.5</td>
<td>Growth disturbances</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>8-9</td>
<td>36-40</td>
<td>Ocular and cerebral malformations</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>25</td>
<td>Growth disturbances</td>
<td>365</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>50</td>
<td>Increase in resorption frequency</td>
<td>364</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>12.5</td>
<td>Ocular and cerebral malformations</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>100</td>
<td>Heart and aortic, face, and urinary tract malformations</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>50</td>
<td>Brain and spinal cord malformations</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>25</td>
<td>Microphthalmia, anophthalmia</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>16-22</td>
<td>10-40</td>
<td>Permanent alterations of nerve cells and cortical architecture of the brain</td>
<td>120</td>
</tr>
</tbody>
</table>

701
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 microscopical 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 60Co 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 pathogenesis.

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 Saylor (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 dd/Y 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 on 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.e., 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 \(2 \times 10^5\)), 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.e. or 6-13 p.e. were compared with other groups receiving a second series of doses on day 14-18 p.e. The foetuses were examined on day 18 p.e. 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, Brizzi 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.e. 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.e. with single whole-body exposures of 12.5-200 R. A second group received 100 R on day 13 p.e. 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.e., 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.e., 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 DNA, 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 protrusion reduced the resorption and the growth retardation 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, 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 60Co 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 unilateral 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 hypoglycaemia 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 cysteaminine (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 cysteamine 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 cysteamine (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. Kallinna (140) tested the effects of a number of possible radioprotective substances, such as phenazine, 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 cysteaminine 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 cysteamine, 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 analyzing 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 inter-comparisons 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 $10^{-2} \text{ R}^{-1}$. 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 preand 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 ontogenetic 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 and 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 regimes 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.
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