7. Chromosome aberrations in atomic bomb survivors of Hiroshima

250. In earlier investigations on the somatic chromosomes of atomic bomb survivors of Hiroshima and Nagasaki, it was shown that cells with radiation-induced chromosome aberrations persisted among the circulating lymphocytes for at least three decades after radiation exposure and that the frequency of aberrant cells was in general proportional to dose. The majority of such aberrant cells were identified as having symmetrical exchanges (reciprocal translocations and inversions) while the frequency of unstable aberrations (dicentrics and rings) was an order of magnitude less frequent [A20, A21].

251. Sofuni et al. [S52] made a further analysis of radiation-induced chromosome aberrations in atomic bomb survivors by comparing the results derived from a conventional staining and a trypsin-Giemsa banding method. Twenty-three atomic bomb survivors from Hiroshima were selected for this investigation. Their estimated doses ranged between 1 to 8.5 Gy of mixed gamma and neutrons; however, these dose estimates are subject to revision. Lymphocyte cultures were established for each individual and the chromosome preparations were made using both the ordinary (O) staining and the trypsin-Giemsa banding method (G). The data are summarized in Table 15 from which it can be seen that:

(a) In general, there is a dose-dependent increase in the frequency of aberrant cells;
(b) The frequencies are clearly higher in the G (banding stain) than in the O (ordinary stain) series. Not shown in the table is the finding that the asymmetrical aberrations constituted only 5% of the total aberrations (17 348 cells identified as having aberration by one or the other method). In addition to easily detectable interchanges involving two breaks, the symmetrical aberrations identified included paracentric inversions, several types of insertion and reciprocal translocations and some quite complex translocations involving more than three breaks.

8. Summary and conclusions

252. Chromosome studies in peripheral blood lymphocytes of people living and working in an area of high natural radioactivity in Austria (radiation burdens from inhaled radon and daughters in addition to external gamma radiation); and of workers exposed to radiation in a number of different occupational settings (nuclear dockyard workers in the United Kingdom, nuclear power plant workers in the Federal Republic of Germany, uranium miners in the United States, workers in the United States with internal depositions of plutonium) have been carried out. Further results from continuing studies of the atomic bomb survivors of Hiroshima have also become available.

253. In the Austrian study, where the different groups studied had accumulated "blood burdens" of 110 to 340 mR a^{-1} of gamma-ray dose and 1 to 1600 mR a^{-1} of alpha dose, the mean frequencies of aberrations (dicentrics, interstitial deletions and fragments) increased with increasing dose, even at these relatively low exposure levels.

254. In the British nuclear dockyard workers (primarily gamma-ray exposures below the maximum permissible limits), significant effects of dose (linear dependence) were evident for the incidence of dicentric aberrations, acentric fragments and cells with unstable aberrations; for all categories of unstable aberrations, the dependence on recent dose was greater (but not significant) than on early dose.

255. In the case of the nuclear power plant workers of the Federal Republic of Germany (external gamma ray and higher energy x-ray exposures; also below maximum permissible limits), although the frequencies of dicentrics and acentrics were significantly higher in the workers than in controls, there was no evidence for a positive correlation between aberration yields and the accumulated total dose even when only the "recent" annual doses were considered. However, in a survey of United Kingdom radiation workers, a linear dose-response relationship was found after appropriate weighting of results to allow for turnover of lymphocytes. The rates of induction of dicentrics and of all unstable aberrations were in reasonable agreement with in vitro dose-response data.

256. In uranium miners in the United States exposure to radon and daughters up to and exceeding 3000 WLM (1 WL is defined as any combination of radon and daughters in 1 litre of mine air which will result in the emission of 1.3 10^{-17} MeV of alpha-particle energy; one WLM is 1 WL times 170 working hours) the frequencies of all aberrations (including inversions, translocations, terminal and interstitial deletions, but excluding dicentrics and rings) showed a dose-dependent increase up to an estimated dose of 3000 WLM.

257. In the case of workers in the United States with internal depositions of plutonium (mean cumulative exposures in the range from 0.03 to 0.33 Sv), there was a significant increase in the frequencies of complex (dicentrics + rings + inversions + translocations) and total (complex aberrations + deletions) aberrations.

258. The results of the study of chromosome aberrations in lymphocytes of the survivors of the atomic bomb in Hiroshima confirm those obtained earlier, namely, a dose-dependent increase in the frequency of aberrant cells (dose range: 1 to 8.5 Gy; mixed neutron and gamma-ray exposures). Furthermore, with the use of Giemsa banding techniques, around 20% more aberrations could be identified, relative to conventional orcein staining technique.

II. EFFECTS IN EXPERIMENTAL MAMMALS AND OTHER SYSTEMS

A. DOMINANT LETHALS AND REPRODUCTIVE CAPACITY

259. The 1977 report considered in some detail the data on the radiation induction of dominant lethals in mice and other experimental mammals. A modest amount of data including those from a comparative study of the effects of 239Pu, fission neutrons and gamma irradiation in male mice have since accumulated; these are reviewed in the following paragraphs.

1. The mouse

260. Grahn et al. [G23] conducted a large-scale study to assess the induction of dominant lethality (among other things) in male mice by incorporated 239Pu and by
external gamma and fission neutron irradiation. Hybrid 16C1F1 male mice 100–120 days old received injections of monomorphic 239Pu citrate in the tail vein at dose levels of 0.19 and 0.37 Mbq/kg body weight. Following the injection, they were mated to 100–150 day old female 16C1F1 mice (1 male x 2 females per week up to 45 weeks). In the neutron series, the male mice were exposed to 0.8 MeV neutrons from the Janus Biomedical Research Reactor (this neutron source has a gamma-ray contamination of less than 3%; single exposures in the range from 0.1 to 1.6 Gy were administered at rates of 0.04 to 0.12 Gy per minute. Weekly exposures (for a total of 6 to 24 weeks) were in the range of 0.008 to 0.13 Gy and were delivered during a 45 minute exposure period each week; the dose rates ranged from 2.10^4 Gy to 3.10^3 Gy min^-1. All doses were midline tissue absorbed doses (MDL) as measured in a tissue equivalent phantom. Dose to the testes may have been up to 10% higher.

261. All gamma irradiation exposures employed 60Co sources. Single gamma irradiation dose levels, in terms of MDL, were in the range of 0.45 to 5.7 Gy and were delivered at 0.3 to 0.4 Gy min^-1. Weekly dose levels of between 0.08 and 2.1 Gy were delivered in a 45 minute period each week with the weekly neutron exposures and dose rates were 2.10^3 to 4.10^3 Gy min^-1. Continuous (22 h d^-1) exposures were given at two MDL levels, 0.03 and 0.06 Gy d^-1 delivered at 2.5 10^-5 and 4.5 10^-5 Gy min^-1.

262. The extent of dominant lethality in the different experiments was assessed by dissecting pregnant females from 10 to 17 days after conception and counting the numbers of corpora lutea, uterine implants (IMP), live implants (LE) early deaths occurring at or around the time of implantation, late deaths and post-implantation losses. However, only the data on post-implantation foetal survival are given in the paper, since the aim of this study was to compare the effects of 239Pu with the other radiations and Pu exposure was not found to influence pre-implantation losses.

263. Concerning first the retention and microdistribution of 239Pu in the testis which was also assayed in this work (a total of 19 mice at the 0.19 Mbq/kg and 23 mice at the 0.37 Mbq/kg levels), it was found that Pu was retained in the testes with no change over the 420-day observation period, with the average retention (both dose levels) being about 0.05% of the initial injected dose. In autoradiographs, about one-half of the alpha tracks were in the interstitial tissue, the remainder occurring in the tubule, mostly originating from plutonium deposited along the basement membrane of the spermatogenic tubule. The heterogeneity of deposition was such that more than 83% of the gonad was radiation-free. In an earlier study from Harwell, Green et al. [G24] examined the inherent heterogeneity of this distribution and concluded that the spermatogenesis within a 10 µm concentric ring inside the basement membrane would receive a dose 2.5 times higher than the whole-organ absorbed dose. Russell and Lindenbaum [R21] have recently confirmed this value and noted that the localized dose to the gonia may be up to 4 times higher than the integrated dose. Initially, a 0.37 Mbq/kg dose produces a total testicular burden of about 5 Bq or about 20 Bq/g equivalent to a daily exposure of about 1.5 10^-3 Gy or about 1 10^-2 Gy per week to the whole gonad.

264. One important observation related to the finding that the testes continued to decrease in weight, while retaining 0.05% of the initial dose. Therefore, the actual dose rate to the gonad would increase with age and duration of exposure. For the purpose of their analysis, the relevant genetic dose had been assumed to be the whole gonad dose and to remain unchanged during the course of the study. This dose was taken to be 1.5 10^-3 Gy d^-1 at 0.37 Mbq/kg and 7.5 10^-4 Gy d^-1 at 0.19 Mbq/kg.

265. The data on plutonium-induced dominant lethals are presented in Table 16. These data were examined for evidence of statistical heterogeneity within mating time sequences; it was found that the weekly variation in LE/IMP proportions were not significant. There was however a dose effect as shown by the difference between the 0.19 and 0.37 Mbq/kg data. A linear regression analysis of these data within and across mating time sequences and within dose levels, demonstrated that the regression of post-implantation survival on time after injection (an indicator for accumulating dose) was not significantly different from zero slope. Therefore, the dominant lethal rate was concluded to be independent of accumulating dose and to principally reflect dose rate. Searle et al. [S33] had discussed these aspects in connection with their 239Pu work and had concluded that the observed dominant lethality was mostly induced in meiotic and post-meiotic cell stages. The gonadal doses for these latter stages of germ cell development were estimated to be 0.021 and 0.042 Gy for the 0.19 and 0.37 Mbq/kg regimes. Under these conditions, the dominant lethal rate could be estimated as (64 ± 11)10^-4 gamete/10^-2 Gy of alpha irradiation to the whole gonad.

266. The results from chronic gamma-irradiation together with estimates of doses are given in Table 17. These data yield a rate of dominant lethality of (5 ± 0.6)10^-4/gamete/10^-2 Gy, for post-meiotic germ cells. The estimates of rates from other data (single and weekly gamma-ray exposures, single and weekly neutron exposures) are summarized in Table 18. Also shown in the Table are the estimates for 239Pu and chronic gamma-irradiation.

267. Inspection of Table 18 will reveal that, for post-meiotic stages:

(a) There are no statistically significant differences in dominant lethal rates for gamma-irradiation delivered either singly or weekly: for chronic gamma-irradiation, however, the rate is only about one-half of the above rates;

(b) There are no significant differences with fission neutron irradiation delivered singly or weekly;

(c) Single gamma-irradiation exposures are more efficient than weekly or chronic exposures in inducing dominant lethality in pre-meiotic stages;

(d) The effects of single or weekly neutron exposures of pre-meiotic stages are not significantly different.

The RBE estimates for these data are given in Table 19 from which it can be seen that alpha irradiation of post-meiotic stages is nearly as efficient as fission neutrons; for pre-meiotic stages, fission neutrons are 19 times more effective than chronic gamma irradiation.

268. In subsequent work, Grahn, Frystak and Lee [G25] have compared the observed dominant lethality in mice to dominant lethality (among other things) by low single doses of neutrons (1 10^-2 to 40 10^-2 Gy) and of gamma rays (0.23 to 1.45 Gy) in male mice. The strain of mice used, age, etc., are the same as those of the study discussed in the preceding paragraphs. The data pertain to the first five
weeks of mating following irradiation. The average number of live implants/pregnant female was slightly reduced and this effect was significant after 0.2 and 0.4 Gy of neutrons and 1.45 Gy of gamma rays. Pre-implantation mortality was slightly increased (significant at all neutron doses except at 0.01 and 0.1 Gy); the increases in the gamma ray series did not reach statistical significance. Post-implantation mortality was slightly higher in high irradiation groups except after 0.01 Gy of neutrons. There were some indications that at the very low doses employed (0.01 and 0.025 Gy of neutrons and 0.23 and 0.45 Gy of gamma rays), post-implantation mortality was higher than expected on the basis of extrapolation from higher doses in earlier work.

269. In an extension of the $^{239}$Pu studies to $^{241}$Am, another transuranic element presumed to have a slightly different metabolic behaviour, Grahn et al. (C26) injected a single dose of $0.37$ MBq/kg of $^{241}$Am (intravenous) and examined the testicular distribution pattern and dominant lethality. It was found that the gonadal retention was only 50 to 60% of that seen for $^{239}$Pu. Retention through the first 100 days after injection remained unchanged (as for plutonium) and preliminary autoradiography indicated that the microdistribution was similar to that seen for plutonium. The estimated testicular dose for the measured burden of about 13 Bq per gm of tissue was 9.2 $10^{-4}$ Gy d$^{-1}$, slightly above the dose delivered by $^{239}$Pu dose of 0.19 MBq/kg. The results of a 10-week dominant lethal series conducted between 125 and 195 days after injection suggested that the amount of intrauterine mortality was low and not significantly different from that in controls.

270. Shevchenko et al. [S133] studied the induction of dominant lethals in mice by incorporated $^{14}$C. Labelled glucose solution (5.4 $10^{4}$ Bq/g, 12.4 $10^{4}$ Bq/g and 25 $10^{4}$ Bq/g) was administered orally to adult hybrid (CBA x C57BL) male mice. The midline absorbed doses in the tests were, respectively, 0.22, 0.5 and 1.0 Gy. It was found that the amount of dominant lethality in post-meiotic germ cells increased linearly with an increase in dose. The authors estimated that the RBE for beta rays from incorporated $^{14}$C (relative to x or gamma rays) was not significantly different from 1.

271. In a series primarily designed to study the induction of autosomal recessive lethals and to assess the length of the sterile period, Lüning et al. [L18] irradiated 60-70-day old CBA male mice with 14 MeV neutrons at 1.5 and 2.5 Gy. The irradiated males were mated to unirradiated females of the same strain (1 male x 3 females) for three consecutive weeks. The pregnant females were killed and examined for intrauterine deaths on the 17th day after the beginning of the matings. The frequency of dead implants was between 8 and 9.5% in the controls (week 1: 9.5%; week 2: 8.5% and week 3, 8.3%). With 1.5 Gy, these frequencies increased from 21.1% (week 1) to 25.3% (week 2) and to 37.8% (week 3). At 2.5 Gy the corresponding figures were: 23.4% (week 2) and 32.4% (week 2) and 49.5% (week 3). At no parallel experiments with x-irradiation were done, no RBE values could be calculated. However, in comparison with previous experiments with the same strain (F12), the authors concluded that 14 MeV neutrons may be between one and two times as effective as x-rays.

272. Yusof [Y9] compared the yields of dominant lethals in caffeine-fed hybrid (C3H x 101) male mice irradiated with 2 Gy of either x-rays (0.7 Gy min$^{-1}$) or $^{60}$Co gamma rays (0.002 Gy min$^{-1}$) and mated to females on either the eighth or fifteenth day after x- and gamma-irradiation, respectively, to sample treated spermatids. The live embryo/corpus luteum ratio in pregnant females killed on about the fourteenth day of pregnancy was 0.51 for x rays and 0.67 for gamma rays. These corresponded to rates of induction of dominant lethals of about 17 $10^{-4}$ and 10 $10^{-4}$ per gamete per $10^{-2}$ Gy, respectively. Caffeine had no significant effect.

273. The above differences in induction rates might have been due to dose rate and/or to quality effects. In order to investigate this, Searle and Beechey [S54] gave (C3H x 101) hybrid male mice doses of 3 Gy of x- or gamma-irradiation at the same high and low dose rates of about 0.6 and 0.002 Gy min$^{-1}$. All litters were conceived 12-16 days post-irradiation and so were derived from treated spermatids as in the previous experiments. Live embryo/corpus luteum ratios were 0.59 and 0.52 for protracted and acute gamma-irradiation, 0.40 and 0.38 for protracted and acute x-irradiation. These ratios correspond to rates of induction of dominant lethals per gamete of 0.39, 0.50, 0.77 and 0.83, respectively. Although for both x- and gamma-irradiation, acute exposures were more effective than protracted ones (protracted/acute ratios of 0.92 and 0.78, respectively), the differences were not significant. However, there were significant quality effects, the relative effectiveness of x- versus gamma-irradiation being 2 at low and 1.7 at high dose rates. In both experiments of Yusof and of Searle and Beechey, the frequencies of abnormal spermatozoa were significantly increased 7 to 8 weeks after irradiation, but there was no consistent effect of radiation intensity or quality. In the experiment of Searle and Beechey, there was no significant effect of dose rate on either testes male or sperm count in the x-ray series, but there was a significant effect in the gamma-ray series, with greater survival at the lower intensity. This result is in line with other evidence which suggests a greater effect of dose rate after gamma-irradiation than after x-irradiation over the same range.

274. Generoso et al. [G27] have recently reported on an experiment designed to examine whether the oocytes in female mice are capable of carrying out repair of genetic damage induced in the male genome. Such experiments although known for a long time in Drosophila (see [S55] for a review) have so far not been carried out in mammals with this specific objective. In the present work, 12-week old males of one stock (either (101 x C3H)F1 or the reverse hybrid (C3H x 101)F1) were irradiated with 5.5 Gy of acute x-rays (or treated with one of four chemicals) and mated to females from different stocks (T, C57BL)(F1), (C3H x 101)F1, and (C3H x C57BL)(F1). Dominant lethality was assessed through dissection of pregnant females in the usual manner, focusing attention on pregnancies that occurred during 0.5 to 3.5 day mating interval (x-ray series), 3.5 to 7.5-day mating interval (1MS), 6.5 to 9.5-day mating interval (TEM), etc. The results showed that, while there was no difference in the yield of dominant lethals in the x-ray series, marked differences were noted in the experiments with chemicals, particularly with 1MS. The frequencies ranged from 9% (C3H x C57BL)(F1), to 50% (C3H x C57BL)(F1), and to 81% (T stock), all at 65 mg/kg body weight (the dominant lethal frequencies were calculated from the ratio of live embryo per pregnant female in the experimental group to those in the controls).

275. Favor et al. [F11] used a modified dominant lethal test to assess the extent to which dominant lethals
were expressed during the later stages of gestation (i.e., late dominant lethals). In this study, 10-week old B6D2F1 hybrid male mice were irradiated with 600 R of x rays and then mated to females of the same genotype and age (1 male × 2 females) for seven days. The females were checked daily for the birth of new litters and the numbers of live and dead at birth were recorded. The progeny were weaned at three weeks of age after which the parental females were sacrificed and their uterine horns examined and scored for live and dead implantation scars according to the method described by Soares [S56, S57]. Unirradiated controls were run concurrently. The data showed that the radiation treatment caused a significant increase in the number of live deaths (defined as the difference between the number of "live scars" and the number of "live born"): the percentage of live deaths increased from a control mean of 7.3% to 28.6% in the irradiated series. The scar method used in this work has the advantage that the females need not be killed at mid pregnancy and that the incidence of dominant lethality can be assessed after the birth of litters.

276. The authors are aware of the fact that the method of assessing live and dead implantations by the scar technique underestimates dead implantations and overestimates live implantations. Using an estimate of error rate (the calculation of which is outlined in their paper) the obtained result by this method can be corrected to be more accurately representative of the live and dead implantations.

277. Goldstein and Spindle [G28] studied the x-ray induction of dominant lethals in male germ cell stages which are expressed from the cleavage stage to the early (trophoblast) outgrowth stages in cultured mouse embryos. Random-bred male mice of the Dubbx(ICR) strain aged 10–12 weeks were irradiated with 4.5 Gy of high dose rate x rays (0.6 Gy min⁻¹) and mated to females of the same strain sequentially (1 male × 1 female twice a week for the first four weeks after irradiation of the males and once a week for the next four weeks). The females, prior to mating, received injection of pregnant mares' serum gonadotrophin and human chorionic gonadotrophin for induction of superovulation. In each group, on day 2 of pregnancy, the females were killed and the oviducts were flushed out to obtain uncleaved and two-celled embryos. The ratio of uncleaved ova to two-celled embryos provided a measure of the fertility. The embryos were cultured and mortality at various stages of development was recorded (Table 20).

278. The data show that:

(a) In the irradiated group, there is a higher incidence of developmental failures in early cleavage, at the late morula stage and at the late blastocyst stage than in the controls;

(b) Dominant lethals are induced more frequently in germ cells exposed as early spermatids and spermatocytes and next most frequently in germ cells exposed as spermatozoa: of the experimental two-cell embryos fertilized 14–28 days after irradiation (spermatids-spermatocytes), 35.9% are arrested during cleavage, primarily at the two- or three-cell stage and this is more than twice the proportion found in controls for the same period. Furthermore, of those fertilized 14–28 days after irradiation that developed to the morula stage, 26.8% did not form blastocyst after 72 h in culture while only 7.8% of the control embryos failed at this stage;

(c) The germ cells irradiated as early spermatids and spermatocytes manifest about equal proportions of dominant lethals at each developmental stage whereas those irradiated as sperm manifest dominant lethality predominantly at the blastocyst. The overall pattern of stage sensitivity as observed in this study agrees well with what has been known from conventional dominant lethal studies [B24].

279. In subsequent work with the same strain of mice and techniques, Goldstein et al. [G29] used four lower x-ray doses (0.9, 1.8, 2.7 and 3.6 Gy). The main observations were the following:

(a) When the germ cells used for fertilization were spermatozoa or spermatids at the time of irradiation, the fertilization index (i.e., the ratio of 2-cell embryos to uncleaved eggs) was not affected at any dose level; with spermatocytes, however, the fertilization index was significantly lower than in the controls at all doses except the lowest one;

(b) With spermatozoa, there was no significant increase in the frequency of embryos arrested in development before blastocyst formation but there was a small, dose-independent increase in these frequencies (between 5 and 9%) after blastocyst formation;

(c) Embryos derived from germ cells irradiated as spermatids showed increased developmental arrest both before and after blastocyst formation; furthermore, the dominant lethals manifesting before blastocyst formation were about equally distributed at all cleavage stages. This latter observation, however, is in contrast to that noted at 4.5 Gy (paragraph 278) by the same workers: at this dose, developmental failures were found primarily at the 2-cell stage. The overall frequencies of dominant lethals over the range 0.9–4.5 Gy gave a satisfactory fit to a model which incorporates a linear and quadratic component.

280. Eiche [E7] conducted a study to examine the effects of low x-ray doses (0.04 and 0.08 Gy) given to young (1-, 2- and 3-week old) female mice and to female foetuses irradiated in utero (at two weeks of age) on intrauterine death. The choice of these groups was dictated by the fact that oocytes in very young females are very sensitive to killing [R22] and the oocytes in the 15-day old foetuses are at a stage when the DNA synthesis is most conspicuous [P21]. Young females or foetuses (CBA × CBA and CBA × Afa) were irradiated and, when 63–64 days old, were mated to CBA or C57BL males. On the 18th day after the beginning of the matings, the animals were killed and examined for intrauterine contents (live foetuses, early deaths and late deaths).

281. The data showed that there were no significant differences in intrauterine death rate between the

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12 Percent dominant lethals:

\[\frac{1 - \left(\frac{1 - \text{fraction arrested at a given stage, experimental}}{1 - \text{fraction arrested at the same stage, control}}\right)}{100}\]

The stage may be early cleavage, late morula or late blastocyst.
control and irradiated groups within any experiment, although the controls in two out of the three experiments gave low values; however, these latter values were within the range of intrauterine death rate of non-irradiated series in earlier experiments from the same laboratory. In all the series of animals in which similar type of females were mated to males of two different strains, there was a considerable difference in the mean number of implants as well as in their distribution. The observation that the mean number of implants per female in the two series in which the females had been irradiated during the first week was considerably lower than in the other series, however, was not borne out in a subsequent study [E8].

282. Baev et al. [B25] exposed adult female mice of the H strain to whole-body chronic gamma-irradiation from a 137Cs source over a 35-day period at a mean dose of about 10 1 GY min⁻¹ (total dose: 5 GY). After the completion of the exposure the animals were mated (2 females × 1 male) to males of the same strain and age for a 7-week period. The females were checked from the 13th mating day onwards and those found to be pregnant were killed and the numbers of corpora lutea, live and dead embryos were counted.

283. It was found that the frequency of pregnant females was lower in the irradiated groups (58%) than in the controls (96%). There was a marked fall in fertility beginning with the second week; from week 3 through week 7, fertility rates varied between 3 and 9% versus 27 and 58% in the controls. Mean corpora lutea counts in the irradiated females were normal at week 1, but declined progressively thereafter through week 7. Counts of implants showed the same pattern of decline, with the rate of decrease even more marked. These data and estimates of dominant lethality [B25] show that:

(a) The induced post-implantation mortality rate is rather low, varying from 2.6 to 6.1% (apart from week 6 where the estimated rate has a negative sign) with no statistically significant difference between the different weeks;

(b) The induced pre-implantation mortality rate, on the other hand, varies over a wide range: (3.1%, week 1; 5.1%, week 2; 15.9%, week 3; 3.2%, week 4; 7.5%, week 5; 17.7%, week 6; 3.9%, week 7; 0.3%, week 8 and 14.9%, week 9);

(c) Consequently, the increase in total dominant lethal rate can be accounted for primarily by pre-implantation loss.

However, the latter (c) is known to include non-genetic effects, such as unfertilized eggs and it is a less reliable index.

284. A comparison of these data with the results of Searle and Beechey [S58] for weeks 1 and 3 after acute x-irradiation exposures in the range of 0-4 Gy (0.7 GY min⁻¹) shows that a decrease in dose rate to 10 1 GY min⁻¹ reduces the rate of post-implantation mortality. The rate observed in the present study with 5 Gy of chronic gamma irradiation is of the same magnitude as that after 1 Gy of acute x-irradiation.

285. Searle et al. [S59] compared the breeding performance of hybrid (C3H/HeH female × 101/H male) F1 female mice given 239Pu (0.19 or 0.37 MBB/kg body mass in 1% trisodium citrate by the tail vein) or kept in a 0.1 GY d⁻¹ or 0.2 Gy d⁻¹ 40Co-gamma-irradiation field for up to six-week periods (but mated in the control area) or unirradiated. The injected (or gamma-irradiated) females and the contemporaneous control females were mated to males of the PT stock. In the plutonium series, most of the females were mated 24 h after injection and were allowed to breed until they failed to produce any live-born litters within two months of the previous one; some other females were killed 24 h after injection or at later intervals for radiochemical or autoradiographic examination. In the gamma-ray series, the mice were initially put together as trios (1 male to 2 females) in the control area and a female was moved into the radiation field when a vaginal copulation plug was found; she was removed to the control area on the 18th day of gestation and the appropriate male added to allow a mating at post-partum oestrus. One day after the birth of the litter (or on the day of birth if a vaginal plug was recorded then) she was returned to the radiation field and only removed when the litter was 18 days old and ready to be weaned. She was then paired with the male again and the procedure was repeated until sterility ensued.

286. The ovarian dose rates from the injected plutonium were initially 8 10⁻³ and 1.7 10⁻² GY d⁻¹, respectively in the two groups, changing little thereafter. Actual gamma-ray dose rates averaged around 8 10⁻³ and 16 10⁻² GY d⁻¹, respectively; the highest dose received was in the first 4-week period 2.6 or 5.2 GY, declining to about two-thirds of these values by the fourth 4-week period. As mentioned earlier, the females were irradiated until the onset of sterility.

287. The results show that both gamma-ray treatments affected the reproductive performance more than the plutonium injections with respect to duration of fertility and offspring per litter in successive 4-week periods, though the overall mean litter sizes were not significantly less than controls. In the gamma-ray series, the percentage of fertile females dropped to zero by the fifth and eighth 4-week periods (respectively, in the 0.2 Gy and 0.1 Gy d⁻¹ groups). In the plutonium series, a similar drop was noticed after 12 and 15 4-week periods, respectively in the 0.37 and 0.19 MBq groups. The mean number of offspring per litter in the gamma-ray series dropped to one-third of that in controls in the 0.1 Gy d⁻¹ group and to one-sixth in the 0.2 Gy d⁻¹ group. In the plutonium groups, this drop was less pronounced reaching only two-thirds of the control level even in the 0.37 MBq group. From these and other data, the authors estimated that the RBE for the effects on reproduction attributed to germ-cell killing is about 2.5 for the alpha particles relative to gamma rays, a value which is lower than that found for testis mass reduction (10-15) in an earlier study [S53]. They suggest that this low RBE may be connected with the inhomogeneity of the alpha-particle dose within the ovary.

2. Other species

288. Chambers and Chapman [C29] studied the induction of pre- and post-natal lethality (among other things) in rats that were given testicular x-ray exposures of either 600 R or 450 R, using litter size and the number of progeny alive after birth at day 1 and at day 21 as criteria. Male rats of the yellow C57 strain were irradiated (either with a single acute exposure of 600 R at 10 weeks of age or with a fractionated exposure of 450 R given in three fractions of 100, 150 and 200 R at 10, 12 and 14 weeks of age, respectively; 85-90 R min⁻¹). Nine weeks after irradiation (a period of time sufficient to ensure that irradiated spermatogonia will be sampled) the males were mated to females and the numbers of progeny born alive and those that survived
until day 1 and day 21 were recorded. The data showed that:

(a) There is a reduction in litter size following spermatogonial irradiation;

(b) Based on the litter sizes in the progeny, the rate of induction of such effects can be estimated to lie between (2.0 ± 1.4)10⁻⁴/gamete/R to (3.0 ± 2.3)10⁻⁴/gamete/R.

These values are in agreement with those calculated by Taylor and Chapman [1] [3] in an earlier study with rats, (1.4 ± 0.8)10⁻⁴/gamete/R, and also with those in mice: Luning's [19] mouse data yield estimates of 1.8 10⁻⁴ and 2.2 10⁻⁴/gamete/R based on litter size at birth and weaning, respectively. Estimates based on the same traits from the mouse data of Lyon et al. [20] are 1.4 10⁻⁴/gamete/R for both traits.

289. Caine and Lyon [30] reported on the effects of x-irradiation on reproductive capacity and dominant lethal induction in female guinea pigs and in the Djungarian hamsters (Phodopus sungorus). The latter species resembles the mouse in body size, is physiologically closer to the hamsters, has a relatively low chromosome number (2n = 28) and is easily maintained under laboratory conditions. Female random-bred, albino guinea pigs and Djungarian hamsters were irradiated with 4 Gy of x-rays at 3–5 months or 8–12 weeks of age, respectively (bilateral irradiation, each side being exposed in turn; 0.5 Gy min⁻¹). The irradiated females were mated to males (guinea pigs: 4 females × 1 male; hamsters: pair-mating) 24 h after irradiation and left to reproduce for two years. Litters being expected at approximately 2–3 month intervals for guinea pigs and 3–4 week intervals for the hamsters. Dominant lethal studies were carried out only with the guinea pig at the first oestrus and at 3 months, while in both species the reproductive capacity was assessed.

290. The following results were obtained:

(a) In the guinea pig, the irradiated females produced fewer offspring in the first litter, but the difference from the controls was not significant;

(b) The mean litter size of irradiated guinea pigs was reduced in the first six months (2.9 versus 4.6 in the controls), but this reduction was barely significant; in the 6–12 month interval and in the remainder of the period there were no differences; there was no significant alteration in the overall mean number of litters per female during the 2-year period, although the irradiated females produced a smaller mean number of offspring than the controls (22.4 versus 31.2 in the controls);

(c) In the guinea pig again, irradiation caused a significant increase in dominant lethals at the first oestrus matings with a lower non-significant induction at three months; the dominant lethal yield at first oestrus matings was due primarily to pre-implantation loss while at three months, post-implantation loss was higher; most of the post-implantation death occurred soon after implantation;

(d) In the Djungarian hamster, irradiation produced a dramatic sterilizing effect, i.e., a lowering of both the total number of litters and litter size: only 12 out of the 48 females in the irradiated group were fertile and these produced only one litter each (in contrast to controls with a mean of 11.8 litters/female) as a consequence of which the total reproductive capacity was drastically reduced (3.3 versus 43.3 offspring per female in the controls).

291. These data demonstrate that the female Djungarian hamsters are at least as sensitive to the sterilizing effect of radiation as the mouse, and considerably more sensitive than the Syrian hamster (Mesocricetus auratus) in which some animals may remain fertile for three months after a dose of 0.5 Gy. The Djungarian hamster thus represents another species for the study of the differences in sensitivity of oocytes to irradiation.

292. In Mikamo's experiments [49], mature female Chinese hamsters were x-irradiated with 50, 100 or 200 R and mated to unirradiated males to assess the amount of dominant lethality in oocytes at the first, third or fifth oestrous cycle after irradiation. The uterine contents of pregnant females were examined at 18.5 days of gestation. There were a total of 436 females (80 controls, 88 for the 50 R group, 121 for the 100 R group and 147 for the 200 R group). The results showed that, with one possible exception, there was no statistically significant increase either in pre-implantation mortality (range: 6.5 to 8.5% in the different irradiation series versus 6.4% in the controls) or in the frequency of abnormal foetuses (range: 8.8 to 10.7% versus 10.8% in the controls). In the third oestrous cycle after 100 R, the frequency of abnormal foetuses was slightly higher (12.9%) and this was correlated with an increase in chromosomal abnormalities in oocytes of females cytologically examined in parallel experiments (see paragraph 378).

3. Summary and conclusions

293. Further data on the radiation-induction of dominant lethals and on the effects of radiation on reproductive capacity have become available from studies with some experimental mammals. Male mice were injected with 239Pu (0.19 and 0.37 MBq kg⁻¹) and mated at weekly intervals to unirradiated females and the amount of dominant lethality was assessed by dissecting the pregnant females at mid-pregnancy and scoring live and dead implants. It was found that the weekly variation in the ratio of live implants to the total number of Implants did not show significant differences for the different weeks, but there was a dose-effect as shown by the differences between the 0.19 and 0.37 MBq kg⁻¹ data (estimated gonadal doses of 0.021 and 0.042 Gy). The amount of dominant lethality observed (mostly induced in meiotic and post-meiotic germ cell stages) was consistent with a rate of 64 10⁻⁴ per gamete per 10⁻² Gy.

294. In the gamma-ray experiments, the rates of dominant lethality were nearly the same irrespective of whether the exposures were administered singly (in the range of 0.45 to 5.7 Gy) or as weekly doses of between 0.08 and 2.1 Gy (sampling of post-meiotic male germ cell stages); for chronic exposures (at the rate of 3.36 10⁻² Gy d⁻¹ and 5.98 10⁻² Gy d⁻¹ with total accumulated exposures of between 0.59 and 1.06 Gy and between 1.05 and 1.89 Gy), the rate of induction of dominant lethals is 5 10⁻⁴ per gamete per 10⁻² Gy which is one-half of that after single or weekly exposures. However, single gamma-ray exposures were more efficient than weekly or chronic exposures in inducing dominant lethality in pre-meiotic stages. The rates (per gamete per 10⁻² Gy) are, respectively, 1.1 10⁻⁴, 0.35 10⁻⁴ and 0.14 10⁻⁴.

295. With fission neutron irradiation of male mice, there were no significant differences in rates in post-
meiotic cells between single exposures (in the range from 0.1 to 1.6 Gy) or weekly exposures (in the range from 0.008 to 0.13 Gy for a total of 6–24 weeks). Furthermore, the effects of single or weekly neutron exposures of pre-meiotic stages were not significantly different.

296. In terms of relative biological effectiveness, alpha-irradiation of post-meiotic stages is nearly as efficient as fission neutrons; for pre-meiotic stages, fission neutrons are 19 times more effective than chronic gamma-irradiation.

297. With low single doses of neutrons (2 to 40 10⁻² Gy) and of gamma rays (0.23 to 1.45 Gy) to males (sampling of germ cells during the first five weeks), there were slight but measurable increases in post-implantation mortality.

298. Fourteen MeV neutrons (1.5 and 2.5 Gy) may be between one and two times as effective as x-rays in inducing dominant lethals in post-meiotic male germ cell stages.

299. The frequency of dominant lethals induced in spermatids by acute x-irradiation (2 Gy) is 1.7 times that induced by protracted (0.002 Gy min⁻¹) gamma-irradiation. Pre-treatment of the males with caffeine prior to irradiation did not affect the dominant lethal yields with either kind of irradiation. Irradiation of spermatids (3 Gy) with low dose rate (0.002 Gy min⁻¹) or high dose rate (0.6 Gy min⁻¹) x-rays or gamma rays showed that the relative effectiveness of x-rays versus gamma-irradiation was 1.9 at low and 1.6 at high dose rates. There were no statistically significant effects of dose rate on testes weight or sperm count in the x-ray series, but there were significantly less severe effects on both with pre-treatment of the gamma-irradiation.

300. The extent to which oocytes in female mice are capable of carrying out repair of genetic damage induced in the males (sampling of mutagenized spermatids) has been investigated by irradiating (or treating with one of four chemicals) males of a given strain and mating them to females of different strains and assessing the amount of dominant lethality. It was found that, while with x-irradiation, there were no significant differences between the different female strains, with chemicals, particularly with isopropyl methane sulphonate, the frequencies of dominant lethals were markedly different with certain strains of females.

301. In one study with mice, a modified dominant lethal method was used to assess the extent to which dominant lethals were expressed during the late stages of gestation. The results (600 R of x-rays; sampling of spermatids) showed that radiation caused a significant increase in the number of late deaths. In experiments designed to examine x-ray induction of dominant lethals in male germ cell stages of the mouse which are expressed from cleavage stages to the early trophoblast outgrowth stages, it was found that the stage at which the embryos die (early cleavage, late morula, late blastocyst) varied depending on the radiation dose and the germ cell stages that were sampled; for instance, with 4.5 Gy, embryos derived from germ cells that were spermatids at the time of irradiation showed developmental arrest primarily at the 2-cell stage. With lower doses, however, (0.9 to 3.6 Gy) and with sampling of the same germ cell stages, the dominant lethals manifesting before and after blastocyst formation were about equally distributed at all cleavage stages. The overall pattern of stage sensitivity as observed in this study agrees well with what has been known from conventional dominant lethal studies.

302. When adult females were exposed to chronic gamma-irradiation over a 35-day period (total dose: 5 Gy), mated to males and the pregnant females analysed for intra-uterine mortality of the embryos, it was found that the induced post-implantation mortality was rather low (2.6 to 6.1%) in the different mating weeks; the induced pre-implantation mortality varied over a range from 0.3% to 18% in the different weeks. The increase in dominant lethality can thus be accounted for primarily by increase in pre-implantation losses.

303. When female mice were injected with ²³⁹Pu or chronically irradiated with gamma rays and then tested for breeding performance, it was found that there was an adverse effect with respect to the duration of fertility and the number of offspring per litter, although the overall mean litter sizes were not significantly less than in the controls. In the gamma-ray series (0.1 and 0.2 Gy d⁻¹ for up to six 4-week periods), the percentage of fertile females dropped to zero by the fifth and eighth 4-week periods (respectively in the 0.2 and 0.1 Gy d⁻¹ groups). In the plutonium series, a similar drop was noticed after the twelfth and fifteenth 4-week periods, respectively, in the 0.37 MBq and 0.19 MBq groups. From these and other data, it has been estimated that the RBE for the effects on reproduction attributed to germ cell killing is about 2.5 for alpha-particles relative to chronic gamma rays.

304. When rats were given testicular exposures of 450 and 600 R and germ cells irradiated as spermatogonia were sampled, there was a significant reduction in litter size and this was consistent with a rate of induction of such effects of between 2 and 3 10⁻⁴/gamete/R.

305. Irradiated (4 Gy of x rays) female guinea pigs produced fewer offspring in the first litter and the mean litter sizes were slightly reduced in the first six months; from this time onwards up to two years, there was no pronounced alteration in the overall mean number of litters per female, but the irradiated females produced a smaller mean number of offspring than controls.

306. In the female guinea pig, irradiation caused a significant increase in dominant lethals at the first oestrus matings, one that was mainly due to an increase in pre-implantation losses. Post-implantation losses were higher 3 months after irradiation.

307. In the female Djurgarian hamster, x-irradiation (4 Gy) produced a marked sterilizing effect, i.e., a lowering of both the total number of litters and litter sizes.

308. In experiments involving irradiation of mature female Chinese hamsters (50–200 R) it was found that the amount of dominant lethality in oocytes at the first, third or fifth oestrus cycle matings was not significantly increased, relative to controls, with one exception: in the third oestrus cycle after 100 R, the frequency of abnormal foetuses was slightly higher relative to controls and this was correlated with an increase in chromosomal abnormalities in oocytes of females cytologically studied in parallel experiments.
B. TRANSLocations

1. Introduction

309. Since the publication of the 1977 report, some new data have become available on the radiation-induction of translocations and other exchange type aberrations in male and female germ cells of the mouse and in spermatogonia of the rhesus monkey. These data pertain to the induction of reciprocal translocations in spermatogonia following irradiation of males (and analysed cytogenetically in descendant spermatocytes), of chromatic aberrations in meiotic stages of male and in oocytes of female mice (also analysed cytologically), of heritable translocations in females (studied using genetic techniques and subsequently verified using cytological methods) and of reciprocal translocations in spermatogonia of the rhesus monkeys (studied cytologically in descendant spermatocytes).

310. Ford et al. [F14] compiled most of the relevant cytogenetic data that bear on the induction of reciprocal translocations in mouse spermatogonia (eight different studies involving examination of a total of over 70,000 spermatocyte preparations of irradiated males and examination of spermatocyte preparations of 681 sons of irradiated fathers). In one of the two studies [S113] involving cytogenetic analysis of spermatocytes of 531 sons of irradiated fathers (spermatoozial irradiation), one male progeny heterozygous for an induced reciprocal translocation was also a mosaic for a Robertsonian translocation: about half of the spermatocytes examined contained a typical "Robertsonian trivalent" which was replaced by two normal bivalents in the remainder.

311. Ford et al. point out that mosaicism indicates that the Robertsonian translocation would have originated during embryonic development, possibly as early as the first cleavage division and that this was presumably a spontaneous event. Apart from this, there were no instances in any of the other studies for the radiation-induction of Robertsonian translocations. The authors point out that, although it is likely that some cases of Robertsonian translocations could have been misclassified as reciprocal ones on the basis of metaphase I configurations (and all the investigations referred to in their paper were carried out before the C-banding procedure was introduced), the number of Robertsonian translocations so missed could have been only very few. As they stated "... it can be concluded with confidence that if Robertsonian translocations are induced by ionizing radiation at all, they are formed with negligible frequency compared to reciprocal translocations".

2. Reciprocal translocations in male germ cells of the mouse

(a) Spermatogonia

312. Breuven et al. [B26] compared the yields of translocations obtained at low exposure rates of $^{60}$Co gamma-irradiation in the range of 1-0.001 R min$^{-1}$ (range: 100-800 R; spermatogonial irradiation) with those after X-irradiation at 100 R min$^{-1}$. Adult CD1 male mice which were 8-10 weeks old at the start of the irradiations were used throughout the study with the exception of a few animals that were 40 weeks old which served as an aged control. The animals were killed at different times after the end of the irradiations depending on the exposure and exposure rate and the tests were processed for cytological preparations.

313. The data are presented in Table 21 and show that the yield of translocation decreases over the range of 100-0.003 R min$^{-1}$ with no significant difference between 0.001 and 0.003 R min$^{-1}$. These data are very similar to those of Pomerantzeva et al. [P22, P23] and Searle et al. [S53] discussed in the 1977 report and permit the conclusion that there is no increase in translocation yield at very low exposure rates. The rate per R at the high exposure rate of 100 R min$^{-1}$ is about 16 times that at the lowest rate of 0.001 R min$^{-1}$ when a correction is made for the relative biological efficiency of gamma rays relative to X rays. The finding of a lack of increase in the rate of translocation recovery at very low exposure rates is also qualitatively in line with that from specific locus experiments [R23, R24] discussed in the 1977 report.

314. In the work with plutonium alpha rays, gamma rays and neutrons reported in the section on dominant lethals, Grahn et al. [G23] also studied the induction of reciprocal translocations in spermatogonia. Tests preparations were made from 8 to 50 weeks after injection of plutonium and the spermatocytes at metaphase I were screened for translocations. The frequencies of translocation configurations observed were such that when they were plotted against total accumulated dose (minus the 13 days of meiosis preceding metaphase I) no dose-effect relationship was evident. However, there was a suggestive indication that a peak response may be obtained at 0.15-0.20 Gy and this possibility is being tested at present.

315. In contrast, weekly doses of fission neutrons up to total doses of 1.2 Gy produce a linear response with a slope of $(6.8 \pm 0.6) \times 10^{-4}$ per $10^2$ Gy for cells with translocations. The response to single doses of neutrons was non-linear and was best fitted with a power function with a coefficient of $0.79 \pm 0.04$. For gamma rays, the weighted linear regression coefficients for the effects of single weekly and chronic gamma irradiations are $(1.53 \pm 0.074) \times 10^{-4}$, $(0.76 \pm 0.077) \times 10^{-4}$ and $(0.175 \pm 0.017) \times 10^{-4}$, respectively. The decrease in effectiveness with decreasing dose rate is consistent with the studies of Searle et al. [S53]. The lowest coefficient from the chronic exposure data is almost identical to the values recorded by Searle [S53] and Pomerantzeva et al. [P22, P23] for comparable low dose rate exposures.

316. In their other study discussed earlier, Grahn, Frystak and Lee [G25] obtained suggestive evidence for translocation induction after single neutron doses of 0.025 to 0.40 Gy and 0.225 to 1.45 Gy of gamma rays. The increases were small but significant at all doses employed except at $10^2$ Gy of neutrons.

317. In the same work reported in the section on dominant lethals, Shevchenko et al. [S133] also obtained cytogenetic data on the induction of reciprocal translocations in mouse spermatogonia by incorporated $^{14}$C. The frequencies were $0.34\%$, $0.84\%$ and $0.66\%$ at the estimated absorbed doses of 0.22, 0.5 and 1.01 Gy, respectively. Comparing the yields of translocations recorded in this study with those published in the literature (chronic gamma irradiation experiments), the authors concluded that the RBE value for $^{14}$C beta rays is about 1.

318. In the study of Yusof [Y9] discussed in the section on dominant lethals, translocations were also
scored in the spermatocytes (descended from irradiated spermatogonia; 2 Gy of high dose rate x rays or 2 Gy of 60Co gamma rays at low dose rate) of males which had been given 0.1% caffeine in their drinking water for eight weeks. As expected, the frequencies of translocations were significantly lower in the gamma-irradiation group (2.8% versus 5.2% in the x-ray group). Caffeine treatment did not have any measurable effect, but in the gamma-ray group, the caffeine-treated males tended to have a somewhat higher incidence of translocations, although the effect was non-significant. The frequency of autosomal univalents was higher after acute x-irradiation than after gamma-irradiation and the univalent frequency tended to be lower in the irradiated groups that had received caffeine (2.9% versus 5.6%, x rays and 1.7% versus 2.0%, gamma rays).

319. In the 1977 report, the work of Cattanach et al. [C32] on the induction of translocations in mouse spermatogonia by fractionated (unequal fractions) x-ray exposures was discussed. In these experiments, the yield of translocations depended on the size and sequence of the exposure fractions. Thus, when a 1000 R exposure was administered as 100 R followed by 900 R 24 h later, the yield of translocations (22%) was similar to that which can be obtained by extrapolation from lower exposures (assuming linearity) and also to that after a 500 + 500 exposure, 24 h apart. However, when the 900 R exposure preceded the 100 R exposure, the response was much lower (7.4%) yet still higher than that produced by a single 1000 R exposure (4.5%). The same order of effectiveness was observed for the length of the sterile period.

320. From these results, the authors concluded that 24 h after the initial exposure the surviving stem cells are more sensitive than earlier, both to killing and to the induction of translocations; and they are no longer heterogeneous in their radiosensitivities so that increasing yields of translocations may be obtained with increasing exposures. Evidence for this loss of heterogeneity came from the observation that numbers of spermatocytes with 0, 1, 2... translocations gave a good fit to a Poisson distribution in the 100 + 500 R, 100 + 700 R, and 100 + 900 R series, but often showed an excess of cells with two or more translocations (suggesting heterogeneity with a more sensitive sub-population) in other series [C33].

321. In further experiments aimed at studying the radiation-induced loss of heterogeneity of the spermatogonial stem cell response to the induction of translocations, Cattanach and Crocker [C33] obtained results at two further exposure levels, namely at 600 and 800 R. The main observation was that the yield of translocations was highest after fractionation (24 h interval) when the treatment regime comprised the 100 R as the "conditioning" exposure followed by the larger "challenging" exposure. The results thus substantiate the earlier observations [C32] and also support the 100 + 500 R data of van Buul and Léonard [B27] in showing that the elevated response also occurs at lower exposures. All these data are thus consistent with the thesis that the stem cell spermatogonia surviving a radiation exposure become sensitized to genetic damage 24 h later and at this time, little of the original heterogeneity in radiosensitivity typical of the unirradiated testis remains [C32, P24].

322. In another study, Cattanach et al. [C63] reported that if the chemical mutagen triethylenuemelaine (TEM) was given to male mice as a "conditioning" exposure, followed 24 hours later by 900 R of x rays, then high translocation yields were obtained as with 100 + 500 R. TEM + 500 R 24 hours later gave a small but non-significant increase above normal response to 500 R, while TEM + 500 R four days later gave a sub-additive yield (as did the reverse order treatment), as had 100 + 500 R five days later in a previous study [C64]. The authors thought it unlikely that TEM and x rays would both synchronize the cell cycle of surviving stem cell spermatogonia in a similar fashion.

323. Cattanach et al. [C63] therefore postulated that the common mediating cause was the depletion of the stem-cell population, probably by preferential killing of cells actually in cycle [C65]. This might have "triggered" the long cycling survivors from a static out-of-cycle condition or G0 phase which may be highly radio-resistant [C65] into a more active and shorter cell cycle (see also [P24]). The authors consider that 24-48 hours later, a very high proportion of these cells may have been synchronously brought into either a sensitive stage of the cell cycle proper or a sensitive longer lasting transitional phase, before having begun a series of rapid cell divisions to repopulate the germinal epithelium. The sharply reduced translocation yields with the fractionation intervals of 3-16 days after 500 R may have then typified the response of the rapidly cycling cells, which seems similar to that found in the actively proliferating mouse testes [C65].

324. In an extension of their earlier study [B27], van Buul and Léonard [B28] gave unequal fractionated x-ray exposures to male mice (spermatogonial irradiation) to define the magnitude of the conditioning exposure that would sensitize the A2 spermatogonia to the challenging exposure 24 h later. In this work, a total exposure of 1000 R was administered in the following way: 100 + 900 R; 75 + 925 R; 50 + 950 R and 25 + 975 R. Appropriate single exposure controls were done concurrently. The results showed, however, that when the conditioning exposure is below 100 R, there was no enhancement of the translocation frequencies. The frequencies recorded were the following: 25R: 0.4%; 50 R: 0.76%; 100 R: 1.3%; 1000 R: 2.5%; 25 + 975 R: 3.3%; 50 + 950 R: 5.0%; 75 + 925 R: 5.0% and 100 + 900 R: 16.1%. These results support the idea that depletion of the stem-cell population is the important cause in triggering survivors into the more active cycle as has been postulated by Cattanach et al. [C63] because at exposures below 100 R, stem cell depletion would be relatively slight though there would still be major killing effects upon differentiating spermatagonia.

325. Van Buul et al. [B29, B69] studied the response of four different stocks of mice (with the Swiss random-bred genetic background (Cpb(SE)SJ) to the x-ray-induction of reciprocal translocations in their spermatogonia. The stocks were the following:

(a) Normal mice;
(b) Mice heterozygous for a reciprocal translocation involving chromosomes I and 13;
(c) Mice that were trisomic for the translocated chromosome but with normal phenotypic appearance;
(d) Mice that were trisomic for the same translocated chromosome, but characterized by severe underdevelopment, skull malformations and abnormal growth of the upper and lower incisors ("teeth trisomics").

326. In the first experiment (which involved the first three stocks) the irradiation exposure was 3 Gy. The
frequencies of translocation recorded were: 8.8 ± 3.5% (normal mice); 6.1 ± 1.9% (translocation heterozygotes) and 9.6 ± 2.6% (normal trisomic mice) showing that while the normal and trisomic mice have similar sensitivities, the translocation heterozygote may have a lower sensitivity. The latter observation has been confirmed in experiments involving 2.5 and 3.0 + 5.0 Gy (24 h interval) in which it was found that the translocation heterozygotes had a lower sensitivity than normal mice (11.4% versus 19.7%, 700 cells scored in each and 24.9% versus 33.8%, 800 cells scored in each). In the second experiment (with a different radiation set-up) in which the comparison was between normal and "teeth trisomic" mice after 3 Gy, the frequencies of translocations were 5.8 ± 2.3% in the normal mice and 5.6 ± 2.2% in the latter showing no significant difference.

327. In another study, van Buul [B30] examined the radiosensitivity of male mice descended from a mouse population which had been exposed to 2 Gy of whole-body x-irradiation every generation (at 26 ± 2 days after weaning) for 69 generations. Non-irradiated male descendants of the 69th generation were obtained from Spalding and at the age of 12 weeks were given a whole-body 400 R x-ray exposure. Control mice of the same strain without radiation history received the same x-ray exposure. After 10-13 weeks, the mice were killed and meiotic chromosome preparations were made. The results showed that the frequencies were the same in both the groups (8.7 and 8.8%) as were the nature of the translocation configurations. The ratio of ring versus chain configurations was somewhat higher in the group with radiation history (1.5) relative to controls (0.9). However, the mean chiasma frequencies were similar in both groups. Van Buul concluded that the radiation history did not alter the radiosensitivity of the males to the x-ray induction of reciprocal translocations in their spermatogonia. These results are in agreement with those reported by Sheridan [S60, S61] in the mouse and by Sankaranarayanan [S62] in Drosophila.

(b) Meiotic stages

328. Adler [A23] investigated the sensitivity pattern of different stages of meiotic prophase of spermatocytes for gamma-ray-induced chromatid aberrations. Ten- to twelve-week-old hybrid male mice (C3H × 101) were irradiated with 300 R of gamma rays (60 R min⁻¹) and primary spermatocytes were sampled on day 1 (diplotene), 5 (mid-pachytene), 9 (zygotene) and 11 (leptotene). The results show that:

(a) Zygote is the most sensitive stage for the induction of rearrangements, the rank order being zygote > pachytene > diplotene > leptotene;
(b) The frequencies of fragments as well as those of autosomal and sex-chromosomal univalents did not vary significantly between the different stages;
(c) At zygote, the exposure-frequency relationship for chromatid interchanges was consistent with a more-than-linear increase with exposure (Y = aD + bD²) and this was also true for fragments.

329. These results are at variance with some others published in the literature. For instance, Tsuchida et al. [T14] found that with 300 R of gamma-irradiation at a lower exposure rate (29 R min⁻¹), diplotene and pachytene were equally sensitive to the induction of rearrangements, but the pachytene stage was less sensitive than diplotene (10.5% versus 16.0%) for the induction of fragments and breaks outnumbered rearrangements. The picture that emerges from the work of Walker [W19] shows that radiosensitivity progressively increases during prophase, diakinesis being the most sensitive (this stage was not investigated in the work of Adler) with respect to both rearrangements and fragments. Her work also showed that acute x-irradiation (0.72 Gy min⁻¹) and protracted gamma irradiation (2 Gy, 10⁻⁴ Gy min⁻¹ over 13 d) were equally effective in inducing structural aberrations if the mean frequencies were weighted for each stage with respect to their time span.

(c) Relationship between partial sterility of translocation heterozygotes and the length of the translocated segment

330. It has long been known that the degree of partial sterility of male translocation heterozygotes may vary from substantially lower than 50% to substantially higher than 50% of normal fertility. The former is mainly dependent upon the proportions in which balanced and unbalanced gametes are represented in the ejaculate, which in turn is a function of meiotic segregation. On the other hand, the formation of multivalent associations observed in meiotic preparations is assumed to be dependent upon the size of the translocated chromosome segment, which influences the possibility of chiasma formation between the translocated segment and its homologous segment in the intact chromosome.

331. Generoso et al. [G41] tested the possibility that the degree of partial sterility of a male translocation heterozygote is correlated with the frequency at which multivalents are observed in the diakinesis/metaphase 1 spermatocytes. The index of partial sterility used is the percentage of dead implants in females that have been mated to translocation heterozygotes. Only males that carried single reciprocal translocations were included in the analysis (45 males identified from analysis of 25 metaphase 1 spermatocytes). The percentage of dead implants was based upon 6 pregnancies from each of 41 males, 9 pregnancies from each of 2 males, 5 pregnancies from 1 male and 4 pregnancies from another. The percentage of multivalents was determined from 25 cells scored for each male.

332. The results demonstrate a significant positive correlation between the degree of partial sterility and the frequency of multivalents in meiotic preparations; this means that the length of the translocated segment has some influence on the proportion of unbalanced gametes in the ejaculate. When the translocated segment is short, the probability of scoring a cell with multivalent is reduced, but the proportion of sperm in the ejaculate with balanced translocation is increased. The reverse is true when the translocated segment is long. What this means is that cytological scoring may be biased in favour of translocations with translocated segments which have a lower probability of transmission. However, it should also be remembered that if the translocated segment is short, there is more chance of post-natal survival of unbalanced zygotes.

3. Heritable reciprocal translocations in female mice

333. In the 1977 report, the results of Searle and Beechey [S63] and of Krishna and Generoso [K18] on the x-ray induction of heritable reciprocal translocations in female mice were presented. In the study of Searle and Beechey, none out of 386 sons of females
given 3 Gy of x rays showed evidence of translocation heterozygosity, although there were 3 confirmed translocations in 294 female progeny. With the same radiation exposure (300 R), Krishna and Generoso found that 4 out of 800 male progeny were partially sterile (and subsequently confirmed cytologically as translocation carriers) and 2 were sterile (and were cytologically normal).

334. The testing of the female progeny from the same experiment has now been completed [K19]. The results show that out of 935 sons tested, 4 are partially sterile (and have been shown to be translocation heterozygotes through cytological analysis), 2 fully sterile (cytologically normal): in addition, 1 daughter with an XO constitution is also semi-sterile. Besides, 7 XO females and 1 XO/XX mosaic have been recovered. These results demonstrate that reciprocal translocations can be recovered in both male and female progeny of irradiated females. If one restricts attention to semi-sterile males alone, a total of 8 translocations have been recovered among 1735 progeny (males + females) which gives a frequency of 0.46% or a rate of 0.15 $10^{-4}$ per R per gamete, about one-half of that after spermatozogonial x irradiation [U1]. The control rate in the work of these authors is 1 in 4392 [G40] such that correction for controls will not affect the rate appreciably.

4. Chromatid-injuries and other aberrations in mouse and Chinese hamster oocytes: in vitro studies

335. Other studies on aberration induction in oocytes have used the technique of culturing oocytes in vitro. In these, at different times after irradiation, the female mice are killed, the oocytes with germinal vesicles recovered and cultured in vitro and then screened for aberrations in metaphase I. The general findings were that:

(a) The frequency of chromosomally abnormal oocytes increased with time after irradiation;
(b) The variation in the yield of aberrations as a function of time between irradiation and ovulation agreed well with the variation in sensitivity to dominant lethal induction over the same period;
(c) From the frequency of aberrations observed in the oocytes, it was possible to predict the frequency of transmissible reciprocal translocations. Studies of this kind reported in the 1977 report included among others, those of Brewen et al. [B31] and of Searle and Beechey [S63].

336. Since then, the results of three studies on the same general problem have been published. Caine and Lyon [C34] conducted experiments to compare the effects of x rays and some chemical mutagens on the yields of aberrations. The x-ray dose was 4 Gy and was given to mature females that were 8-12 weeks old. The oocytes were recovered after one and three weeks, cultured in vitro and processed for examination of aberrations. The results confirm the earlier findings, namely, that the yield of aberrations is significantly higher in week 3 than in week 1 and this is true of the proportion of abnormal cells in the two sampling times (59.5% versus 41.5%). The aberrations studied included chromatid gaps and breaks, isochromatid gaps, fragments and rearrangements. Fragments, usually isochromatid, were the most common aberration type and more aberrations were found in week 3 (all four categories) with the frequency of rearrangements being more than twice that in the first week (22% versus 8.5%).

337. Brewen and Payne [B32] conducted a study to analyse in detail the radiosensitivity of mouse oocytes from the standpoint of the induction of aberrations. Four x-ray exposures (50, 100, 200 and 300 R) were used. Eight- to ten-week-old female mice of the CD1 strain were irradiated and the oocytes were collected at various intervals ranging from 1.5 days to 28.5 days. The types of aberrations scored were chromatid deletions, isochromatid deletions and chromatid interchanges. The results showed that, as expected, the sensitivity was different depending on the degree of maturation of the oocytes: the least sensitive oocytes were those that were 0.5-1.5 days from ovulation (probably corresponding to stages 7 and 8); the sensitivity gradually increased with longer intervals between irradiation and ovulation until a stage of peak sensitivity was reached at 9.5 days. From this time, the aberration yield remained relatively constant until the females became functionally sterile.14

338. The data were analysed by the authors in three ways. Firstly, the data from all time intervals at each exposure were pooled. Secondly, the data from the least sensitive time intervals at each exposure were pooled. Thirdly, the data from the period of uniform sensitivity at each exposure were pooled. Exposure-frequency regression analyses were done on these pooled data and the best fits were to the models Y = a + bX and Y = a + cD2 for both deletions and interchanges (where terms a, b and c correspond to the coefficients of the spontaneous, one-track and two-track terms, respectively). These analyses have thus demonstrated that the aberrations under consideration result from a predominantly two-track process. The data on which these calculations were made are summarized in Table 22.

339. Brewen, Payne and Adler [B33] carried out a study to examine the induction of chromosome aberrations in mouse dietyate oocytes after fractionated x-ray as well as after chronic 60Co gamma-ray exposures using procedures similar to those employed in their earlier work [B31]. In the first series, a total exposure of 400 R of x-rays was split into two fractions separated by time intervals of 90, 135, 180 and 1440 min. In the second, the same total exposure was administered in unequal fractions (100 + 300 R or 300 + 100 R) separated by 90, 135 and 180 minutes. In the third series, chronic gamma-ray exposure was given to female mice over an eight-day period, 9-16 days prior to ovulation and the exposures were 117, 240, 348 and 483 R. Appropriate single exposure controls were concurrently run. The frequencies of interchanges and deletions were determined in metaphase I oocytes.

340. Considering first the results of series 1, it was found that with 90- and 135-minute intervals between fractions, the yields of interchanges did not significantly deviate from those expected on the basis of interaction of chromosome breaks produced by the two fractions (the yield with the 90-minute fractionated interval was identical to that after the single exposure; with the 135-minute interval, it was lower). With intervals of either 180 min or 24 h, the observed frequencies of interchanges were similar but lower than those with shorter intervals between the fractions and

14 The authors have defined functional sterility as the inability to super-ovulate a sufficient number of oocytes to make slides. For instance, after 300 R, only about 35 oocytes were obtained from 100 females at the 28.5-day interval. This degree of oocyte killing makes cytogenetic studies difficult, but does not preclude the possibility of some females being capable of producing a few offspring.
were not significantly different from the expectation based on additivity. With deletions, the frequency was consistent with interaction with the shortest interval (90 minutes) but not at other intervals.

341. The results of the second series of experiments indicate that there was no difference in aberration yield and thus in the rejoining time, irrespective of whether the first fraction was 100 or 300 R. For interchanges for instance, the frequencies were the following: 100 + 300 R, 90 min: (74.7 ± 5.0)%; 300 + 100 R: (62.4 ± 7.1)%; 100 + 300 R, 135 min: (62.8 ± 5.6)%; 300 + 100 R: (61.5 ± 5.1)%; 100 + 300 R, 180 min: (52.9 ± 4.3)%; 300 + 100 R: (52.5 ± 4.3)%. The authors consider that these data "...argue strongly that the mouse oocyte's repair systems, at least for chromosome aberration formation, are not drastically altered by the magnitude of the X-ray dose, and that this repair occurs within hours and not days or weeks".

342. The data from the chronic gamma irradiation series show that the frequencies are much lower than after acute irradiation and this is true for both interchanges and deletions; and the frequencies observed are consistent with a linear increase with exposures. These data demonstrate that when the exposure rate is reduced from 100 R min⁻¹ (X-rays) to 0.04 R min⁻¹ (gamma rays), the yield of exchanges is reduced to one-tenth at a total exposure of 478 R. The magnitude of reduction in the yield observed in this study is lower than that observed in specific loci work involving maturing oocytes. The combined data from the fractionation and low exposure rate exposures led the authors to conclude that the exposure-frequency relationships for both exchanges and deletions induced by X-irradiation are due to the involvement of two-track processes.

343. In the Chinese hamster, Mikamo [M49] conducted a study to assess the chromosomal radiosensitivity of the oocyte stages sampled from X-irradiated 5-month-old mature females. Oocytes sampled from females irradiated at 85, 59, 35 and 19 hours before ovulation (and analysed at metaphase II) manifest a relatively low level of sensitivity to the induction of chromosome aberrations (mainly chromatid breaks from 0.3 to 4.1%). From 17 hours before ovulation, the sensitivity begins to rise (10% breaks) reaching a peak at 11 hours before ovulation (43.5%); the stage of peak sensitivity corresponds to diakinesis just prior to the onset of prometaphase I. Prometaphase I and metaphase I stages (9 and 7 hours before ovulation) retain a relatively high level of sensitivity (aberration frequencies of 31.4 and 25.3%) although this is not as high as diakinesis. The exposure-frequency relationships for chromatid breaks at the most sensitive diakinesis stage appears consistent with a greater than linear increase in yield with increasing exposures (50-200 R range).

5. Reciprocal translocations in spermatogonia of the rhesus monkey and comparison with results from other mammalian species

344. In the discussion of the data on the induction of reciprocal translocations in the spermatogonia of the rhesus monkey in the 1977 report, it was pointed out that:
(a) The frequencies in the rhesus monkey are much lower than in man, marmoset and the mouse;
(b) Peak yields of translocations are obtained at around 1-2 Gy in the monkey, levels that are much lower than in the mouse;
(c) There are significant differences in the response between the different animals even at the same dose level and in the frequencies recorded by Lyon et al. [L23] on the one hand and by van Buul [B34] on the other.

345. Van Buul [B35, B36] has now collected more data on translocation induction in the spermatogonia of the rhesus monkey and these are presented in Table 23 (the translocations were scored in C-banded preparations). It can be seen that:
(a) The spontaneous frequency of translocations in the rhesus monkey is low and comparable to that recorded for other mammals studied so far in this respect such as the mouse, marmoset, rabbit, guinea pig and golden hamster;
(b) The dose-response curve is humped with a maximum around 1 Gy and a humped curve has been found for all mammalian species studied;
(c) The chiasma frequencies in the rhesus monkey are much higher than in the mouse, rabbit, golden hamster, etc., and consequently the ratio of ring versus chain configurations is much higher in the rhesus monkey than in other species.

346. Van Buul [B36] fitted the translocation data to linear and linear-quadratic equations (0.5 and 1 Gy data) and found that these gave a good fit to the linear model $Y = a + bD$ and the fitted values of $a$ and $b$ are, respectively, 0.0233 and 0.0086. A comparison of the values of the slopes for the data of the rhesus monkey and those of other species (mouse, rabbit, guinea pig, marmoset and man) show that the value for the monkey is significantly lower than that of other species except the guinea pig (see also [B68]). These comparisons are summarized in Table 24.

347. In a cytogenetic study, Benova et al. [B79] compared the sensitivities of the spermatogonia of the mouse, rabbit, Syrian hamster and the rat to the gamma-ray induction of reciprocal translocations. The doses were in the range of 0.5 to 5 Gy and the dose rate was either 1.23 Gy min⁻¹ or 10⁻⁴ Gy min⁻¹. It was found that for exposures given at the high dose rate, the sequence (in decreasing order of radiosensitivity) was the following: rat, rabbit, mouse and the Syrian hamster. For low dose rate exposure, the sequence was: rabbit, rat, Syrian hamster and the mouse.

6. Summary and conclusions

348. Reciprocal translocations are the predominant kind of structural aberrations induced by ionizing radiation in mouse spermatogonia. An examination of all the relevant cytogenetic results that bear on the radiation-induction of translocations in mouse spermatogonia support the thesis that, if Robertsonian translocations are induced, they are formed with a negligible frequency relative to reciprocal translocations.

349. In a cytogenetic study, a comparison was made of the yields of reciprocal translocations after spermatogonial irradiation of mice at different exposures and at high and low dose rates (X- and gamma-irradiation). The results showed that the yield per unit dose decreases over the range of exposure rates from 100 to 0.003 R min⁻¹ (a maximum of 16-fold difference) with no further decrease at still lower exposure rates. These
results support those from earlier studies discussed in the 1977 report.

350. With plutonium alpha-ray irradiation of mouse spermatogonia, there are indications that the peak yield of reciprocal translocations may be obtained at doses of between 0.15 and 0.2 Gy. Caffeine treatment of male mice prior to irradiation (spermatogonial X- or gamma-irradiation) does not affect the yield of translocations.

351. A number of additional experiments (subsequent to those reported in the 1977 report) involving irradiation of mouse spermatogonia with unequally fractionated x-ray exposures (100 + 900 R, 100 + 500 R etc., in this or reverse order with a 24-hour interval between the fractions) have been completed. The yield of translocations was found (as has been the case in earlier studies) to depend on the size and sequence of the exposure fractions. With a small fraction preceding a large one, the yield is high and is equal to that linearly extrapolated from lower exposures. With the reversed sequence of exposures, the yield is low, but higher than that after a single acute exposure. These observations have been interpreted on the assumption that 24 hours after the first fraction, the surviving stem cells are in the more sensitive stage than before, both to killing and to translocation induction and that they are less heterogeneous in their radiosensitivities at the time they receive the second fraction (the degree of synchrony is dependent on the magnitude of the first fraction). In other fractionation experiments involving unequal x-ray exposures (100 + 900 R, 40 + 950 R, 25 + 950 R and the single exposures) it was found that when the conditioning exposure was below 100 R, there was no enhancement of translocation yields. These results support the idea that depletion of the stem-cell population is the important factor in triggering survivors into the more active cycle and that at exposures below 100 R, stem-cell depletion would be relatively slight.

352. Heterozygosity for a specific reciprocal translocation involving chromosomes 1 and 13 in mice appears to decrease the radiosensitivity (relative to normal mice) for the x-ray induction of reciprocal translocations in their spermatogonia. But tertiary trisomic mice (trisomic for the same translocated chromosome) however, do not differ in radiosensitivity from normal mice, with respect to the same end-point.

353. When male mice descended from a population that had a previous radiation history (for 69 generations; exposed to 2 Gy per generation at 26 days after weaning) were tested for their radiosensitivity (translocation induction in spermatogonia) and compared with non-irradiated descendants, no differences could be discerned.

354. A study has been conducted to examine whether reciprocal translocations induced in mouse spermatogonia are subject to selection between the stage at which they are induced and the spermatocyte stage at which they are scored. The results are consistent with the view that there is probably no selection against spermatocyte carrying translocations in the exposure range from 50 to 300 R.

355. There are results which document the premise that in male mice which are heterozygous for a reciprocal translocation, there is a significant positive correlation between the degree of partial sterility of these males and the frequency of multivalents in meiotic preparations.

356. The completed studies on the x-ray induction of heritable translocations in female mice demonstrate that reciprocal translocations can be recovered in both male and female progeny of irradiated females and that the rate of 0.15 10-4/R/gamete is about one-half of that after irradiation of spermatogonia.

357. Subsequent to the publication of the 1977 report, further studies on x-ray induction of aberrations in female mice (using the technique of culturing the irradiated oocytes in vitro) have been conducted. The results obtained confirm those discussed in the 1977 report in showing that the frequency of chromosome aberrations in oocytes recovered three weeks after irradiation were significantly higher than those in oocytes recovered in week 1. In another investigation, it was found that the least sensitive oocytes were those that were 0.5–1.5 days from ovulation and that the sensitivity gradually increased, reaching a peak around 9.5 days from ovulation; from this time onwards, the aberration yield remained relatively constant until the females became functionally sterile.

358. Again in studies using in vitro culturing of irradiated mouse oocytes, when the x-ray exposures were fractionated with intervals of between 90 and 180 minutes and of 24 hours between the fractions, it was found that with 90 and 135 minute intervals, the yields of interchanges did not deviate from those expected on the basis of interaction of chromosome breaks produced by the two fractions; with intervals of either 180 minutes or 24 hours, the yields of interchanges were consistent with the additivity expectation. With deletions, the frequency was consistent with interaction with the shortest interval (90 min), but not at other intervals. Similar studies with chronic gamma-irradiation of female mice and scoring for aberrations in their oocytes in vitro demonstrated a pronounced dose rate effect: at an exposure of 478 R, the yield of chromatid interchanges was only one-tenth after chronic than after acute exposures.

359. In the Chinese hamster, oocytes sampled from females irradiated 11 hours before ovulation (and analysed at metaphase II) manifest the highest level of radiosensitivity to the induction of chromosome breaks relative to other stages. The stage of peak sensitivity corresponds to diakinesis prior to the onset of prometaphase. In this stage, the frequencies of chromatid break increase faster than linearly with an increase in x-ray exposures.

360. More extensive data on the induction of reciprocal translocations in the rhesus monkey spermatogonia have now become available. These confirm the conclusion reached in the 1977 report in showing that the rhesus monkey is much less radiosensitive than most of the mammalian species studied in this respect. The peak yield is obtained at around 1 Gy.

C. LOSS OR ADDITION OF CHROMOSOMES: NON-DISJUNCTION

1. Introduction

361. The 1977 report considered the importance of experimental models to study non-disjunction together with the data that were then available in the mouse, mouse-tobacco mouse hybrids and in the northern field vole, Microtus oeconomus. In this section, the information that has accumulated since then will be
discussed and placed in perspective with the earlier work that has been done in this area. Useful reviews on this subject with particular reference to the mouse [R25] and to experimental mammals in general [H39, H40] have been published.

2. Methods to study aneuploidy

362. The methods that have so far been used can be broadly divided into cytogenetic and genetic ones. The cytogenetic methods, which are currently in extensive use in several laboratories, involve chromosome analysis of second meiotic metaphase in oocytes [H41, R26, R27, U10, U11] or spermatocytes [B42, O8, S67]; pre-implantation embryos at the first cleavage stage [D15, F15, F16, K20, M45] or morulae and blastocysts [F17, G30, Y4]; post-implantation embryos [F17, S68, T17, Y4, Y5, Y6]; and newborn offspring (in mice) [G31].

363. Methods for chromosome preparations from oocytes and from pre-implantation cleavage stages involve modifications of Tarkowski's air-drying technique [T18]. The technique of Evans et al. [E10] for early post-implantation embryos has also been modified and "banding techniques" have been employed for a better identification of the chromosomes. Chromosomes from spermatocytes at metaphase II are usually prepared according to the method of Evans et al. [E11]. All these methods make use of direct chromosome analysis to assess the amount of aneuploidy in male or female germ cells or in embryos at various developmental stages.

364. Some of the genetic methods, particularly those aimed at studying sex-chromosomal non-disjunction have been in use since the early 1960s [K21, R25, R28, R29, R30] and involve studying the segregation of sex-linked marker genes in genetic crosses of appropriate stocks. The genetic method described by Lyon et al. [L30] in 1976 using Robertsonian translocations has not yet been employed in any further published study, neither have other possible methods that would use marked autosomes [R25].

3. Spontaneous incidence of aneuploidy

365. Data on the spontaneous incidence of some numerical chromosome anomalies of interest in different mammalian species are summarized in Tables 25 and 26. It can be seen that in most species, the incidence of aneuploidy in early embryos is rather low in contrast to the situation obtained in humans. Not mentioned in the tables is the finding that the incidence of aneuploidy in the first cleavage stages of the embryos varies between different strains of mice and is generally higher when fertilization is effected in vitro than in vivo [F15].

4. The mouse

366. Chandlely and Speed [C35] and Speed and Chandlely [S114] have recently reported their results on x-ray-induced non-disjunction in male and female mice. In the male series, random-bred Q strain adult mice were irradiated with 1 Gy of x rays and mated to females five weeks after irradiation (to sample spermatocytes) or seven weeks after irradiation (to sample spermatogonia). Appropriate unirradiated controls were also run. Pregnant females from week 5 and week 7 and control matings were killed at 9–10 days of gestation and chromosome preparations made from all viable foetuses or their membranes. The data to be discussed below are from the paper of Speed and Chandlely [S114] since the earlier paper [C35] was a preliminary report.

367. In the above investigation, the overall frequency of abnormalities in the controls was 1.1% (6/571) and included 2 trisomies (41,XXX), 1 triploid (60,XXY) and 3 mosaics (39/40); the frequency of trisomies alone is 0.35%. In week 5 of the irradiated series, the frequency of abnormalities was 2.0% (20/1005) and included 2 monosomies (39,X), 1 trisomy (41,XY,+16), 1 triple-trisomy (43,XXY,+10,+17), 5 triploids, 2 tetratoids, 8 mosaics and 1 with miscellaneous aberration (40,XY,1q+). In week 7, the frequency of chromosomally abnormal embryos was slightly higher being, 2.8% (20/688); 2 monosomies, 7 trisomies, 8 mosaics, 1 triploid and 2 tetratoids. The frequencies of trisomies alone were, respectively, 0.20% (2/1005) and 1.0% (7/688) in weeks 5 and 7 and were not significantly different from the control frequency of 0.35%.

368. In the female series, there were three irradiated groups (6–8 week old females irradiated with 5 10-2 Gy; 9 month old females irradiated with the same dose and mated; and 6–8 week old mice irradiated with the same dose and mated when 9 months old) and two controls (young and aged). Techniques for making chromosome preparations were the same as those mentioned earlier.

369. When young females were irradiated, the frequency of chromosomally abnormal embryos was 1.5% (2/129); 1 trisomy and 1 mosaic) and not significantly higher than in controls (1.1%; 6/571; same as given in paragraph 367). When young females were irradiated, aged and then mated, the frequency was 2.1% (3/145; 1 monosomy, 1 trisomy and 1 mosaic); the corresponding frequency for irradiation of aged females was 3.6% (6/168; 3 monosomies, 3 triploids). The frequency of chromosomally abnormal embryos in the aged female control group was also 3.6% (6/168; 1 monosomy, 1 trisomy, 2 mosaics, 1 triploid and 1 tetraploid). Thus, apart from a general increase in the frequency of chromosomally abnormal embryos with an increase in maternal age, there is no measurable effect due to irradiation at this dose level.

370. Max [M63] irradiated virgin female mice (from an inbred CBA strain) of 6, 15 and 46 weeks of age (groups 1, 2 and 3, respectively) with 2, 4, 8 or 16 R of x rays and mated them to young males of the same strain when the irradiated mice were, respectively 16 or 32–35 weeks old (groups 1 and 2). Group 3 mice were mated soon after irradiation. The foetuses were examined for chromosomal abnormalities, particularly aneuploidy. There were concurrent controls. In the controls (all age groups pooled), there were 2 trisomic and 2 triploid embryos out of 213 screened; the former were found in group 3 controls (2/27) while the latter were in group 2 (2/78). In the irradiated series (all radiation groups) there were 1 trisomic and 6 triploid embryos among 642 embryos: the single trisomic was found in group 2 (1/289) and the triploids were in groups 1 (4/307) and in group 2 (2/289). Thus in these experiments, while there is some suggestive evidence for an increase in chromosome abnormalities with increasing maternal age, no radiation effects could be demonstrated.

371. In the experiments of Strausmanis [S68] virgin inbred C57BL females aged eleven months were
irradiated with 4, 8 or 16 R of x rays and placed with young untreated males of the same strain, five days after irradiation. The pregnant females were killed 10 days after vaginal plugs were observed and the conception was processed for chromosome analysis. Aneuploid embryos classified as alive (heart beats observed while dissecting) were 1 monosomic in the control group (out of 496 embryos) and 2 trisomics in the irradiated groups together (out of 568 embryos). The number of aneuploid embryos classified as dead was 4 trisomics in the control and 3 trisomics in the irradiated groups. These data show that trisomic embryos are not uncommon in the mouse but die after implantation. In any case there is no evidence for an increased frequency of chromosome abnormalities in embryos of aged female mice x-irradiated before mating as compared to non-irradiated ones.

372. In the study of Hansmann et al. [H39, H40] female mice of the NMRI/Han strain (8–12 weeks old) were x-irradiated with 20 R or 40 R to unirradiated males. Cytological preparations of foetuses were carried out 9.5 days after detection of vaginal plugs on the morning after irradiation. The results showed no evidence of aneuploidy among 90 karyotyped control foetuses and among 95 embryos in the 20 R series. However, one embryo out of the 22 karyotypes in the 200 R group was monosomic (chromosome 19) and another trisomic (chromosome 17) and these findings were confirmed in all metaphases examined after banding.

373. In another experiment, Hansmann [H39] and Hansmann et al. [H42] irradiated 8–12 week old male mice with 20 or 200 R and mated them from day 2 to day 36 after irradiation to unirradiated females of the same age and strain. Foetal preparations were made on day 9.5 post coitum. Out of 211 karyotyped foetuses in controls, there were no aneuploids. In the 20 R series, there were 3 aneuploids among a total of 216 foetuses analysed (1.4%) and in the 200 R series, there were 7 aneuploids among 385 foetuses analysed (1.8%). The genetic constitutions were (with the origin in terms of the day of mating given in parentheses): 20 R: 41,XX,+11 (21); 41,XY,+2 (21) and 41,XX,+11 (31); 200 R: 39,XXO (4); 39,XYO,−8 (5); 41,XX,+14 (7); 41,XY,+13 (16); 39,XXO (25); 41,XXY (28); 40,XO,+13 (32). It is worthy of note that the induction of aneuploidy is not significantly enhanced by the higher exposure relative to the lower one. Irradiation did not seem to enhance the incidence of polyploidy in this study.

374. In 6 of the 10 observed aneuploid foetuses, the mal-segregation could be allocated to meiosis I or II; the other 4 foetuses, however, were derived from sperm irradiated in the vas deferens (39,XXO and 39,XYO,−8) or the epididymis (41,XX,+14) or irradiated as mid spermaticids (41,XY,+13). A chromosome loss most probably may explain the XO karyotype. Mitotic non-disjunction during early cleavage stages could lead to the two trisomic foetuses (trisomy 13 and trisomy 14) generated 7 and 16 days after irradiation [H42].

375. Russell et al. [R75] have used a genetic method to determine whether advancing age affects non-disjunction in irradiated or unirradiated female mice. Sex-chromosome markers were built into the cross so that maternal non-disjunction (X<sup>MXMY</sup> or OX<sup>P</sup>) could be distinguished from paternal non-disjunction (X<sup>MXPY</sup> or X<sup>MO</sup>) (any XXX types would be only questionably detectable by phenotype; and XO can result from breakage-related chromosome loss as well as from non-disjunction). Irradiated (200 R; x rays; 1 day before mating) or control females ranged in age from 2.5 to 12 months at conception. Over 13 000 offspring have been scored with only one case of maternal trisomy (X<sup>MXMY</sup>/X<sup>MY</sup> mosaic) derived from a 12-month old irradiated female and one of paternal trisomy (X<sup>MXPY</sup>/Y), and no clear-cut differences in either OX<sup>P</sup> or OX<sup>M</sup> frequencies with age.

5. Other species

376. Although the Chinese and Syrian hamsters have been used for the study of oocytes and pre- and post- implantation embryos in recent years [B43, H43, H44], irradiation data are very scanty. The unpublished observations of Hansmann quoted in his paper [H39] show that following x-irradiation of female Chinese hamsters and examination of metaphase II oocytes there is not a group of oocytes irradiated with 20 R or 200 R (147 and 131 oocytes, respectively, were examined) or in the control group (121 oocytes).

377. In the Chinese hamster study of Mikamo [M49] discussed earlier, chromosome analyses were also carried out (at metaphase II) in oocytes of 5-month-old virgin females that had received 50, 100 or 200 R of x-irradiation; of 16–19 month old females irradiated with 50 R. In the first series, in each of the first, third and fifth cycles after irradiation, unfertilized eggs were collected and processed for chromosome analysis at metaphase II; in the second series the oocytes collected were those in the first cycle after irradiation.

378. In the first series, 8197 oocytes from 1200 females were chromosomally analysed (1747 eggs from 254 control animals, 1812 oocytes from 309 females in the 50 R group, 2300 oocytes from 336 females in the 100 R group and 2338 oocytes from 301 females in the 200 R group). The data showed no significant increase in the frequency of aneuploid eggs except in the 200 R first cycle group where there was a slight but significant increase in the frequency of aneuploid eggs (3.4 versus 2.1% in the controls). With respect to structural abnormalities, again there were no significant increases except in one group: in the third cycle eggs of the 100 R group, the frequency of these anomalies was 1.1% versus 0.2% in the controls (see also subsection II.A.2). The author has no explanation for the statistically significant increase in these two particular samples.

379. In the second series (with aged females irradiated with 50 R), there was a demonstrable effect of maternal age with respect to the production of spontaneous aneuploids (4.1% in the aged controls versus 2.1% in the young controls of the first series), but no effect of irradiation was detected in the treated group (4.0%). With respect to structural aberrations, there was no measurable effect of either maternal age or irradiation (0.2% in the young controls, 0.4% in the aged controls, and none in the treated group).

380. The other results obtained by Mikamo [M49] relate to the induction of aneuploidy in oocytes sampled from irradiated Chinese hamster females irradiated at 85 to 7 hours before ovulation. In these experiments (200 R), the frequencies of hyper-haploid eggs fluctuated around a mean of 1.5% (1526 oocytes sampled in 10 different periods before ovulation) with a range from 0% (35, 15 and 7 hours before ovulation) to
3.4% (59 and 11 hours before ovulation). The mean frequency of hypo-haploid oocytes was slightly higher (2.4%) with a range of between 0% (13 hours before ovulation) and 5% (11 hours before ovulation). The control frequencies of hyper- and hypo-haploid oocytes were, respectively, 0.8% and 1.3% (1839 oocytes sampled). In one stage (oocytes sampled 11 hours before ovulation; as will be recalled, this stage showed the highest sensitivity to the induction of chromatin aberrations), which was studied in detail, the data at exposure levels of 50, 100 and 200 R show small but non-significant increases (the latter probably due mainly to the small numbers of oocytes that could be sampled) in the frequencies of hyper- and hypo-haploid oocytes at 50 R (2.1 and 4.2%, respectively; 1/48 and 2/48 versus the control rates of, respectively 0.8 and 1.3%) with no consistent increase at higher exposure levels.

381. In the 1977 report, the preliminary results of Tates et al. [T19] from studies on non-disjunction in the Northern field vole, Microtus oeconomus, were reported. In this species, C-banding techniques have shown that the X chromosomes have large blocks of centromeric heterochromatin and the Y chromosome is C-band positive along its entire length. This permits the identification of spermatids having single Xs, two Xs and two Ys. Using this technique, Tates obtained good evidence for the induction of non-disjunction (in pre-spermatid stages) at x-ray exposures as low as 50 R, but the calculation of the exact frequencies was complicated by the frequent variability between animals within exposure levels and within and between sampling intervals.

382. In their 1979 paper, Tates et al. [T20] presented the results on the x-ray induction of sex-chromosomal non-disjunction and of diploidy induced in pre-spermatid stages of Microtus, and scored cytologically in the descendant spermatids. The following conclusions may be drawn:

(a) In controls, the frequency of non-disjunction spermatids is about one-fifth of that of diploid spermatids (0.5×10⁴ versus 2.6×10⁴);

(b) In irradiated animals (range 0.25 to 2 Gy), particularly in those given relatively high doses, there is a pronounced induction of non-disjunction and of diploid spermatids at most of the sampling times; the exposure-frequency relationships for non-disjunction spermatids show, however, no uniform pattern at the different sampling times. On days 1 and 12, the frequencies increase with increasing exposures, but at days 2, 4 and 8, the pattern is irregular, particularly at lower exposures. The induction of diploid spermatids shows in general a better exposure-frequency relationship;

(c) At any given exposure level, the mean frequencies of non-disjunction spermatids and of diploid spermatids vary at different sampling time;

(d) The induction of non-disjunction spermatids and of diploid spermatids occurs during the first as well as the second reduction division.

383. Tates et al. [T20] noted that the induction of non-disjunction of sex-chromosomes and of diploid spermatids are not necessarily linked, in the sense that sometimes there is a significant induction of one kind of event and not of the other. The usefulness of this system for the study of non-disjunction is to some extent marred by the occurrence of significant heterogeneity between animals, within exposures and between experiments and sampling times.

384. In a relatively large-scale extension of this work involving 4 different x-ray doses (0.25, 0.50, 1.00 and 2 Gy), 6 different sampling times (1, 4, 6, 7, 9 and 12 days after irradiation) and 6 animals per dose point, Tates and de Vogel [T21] were unable to demonstrate any significant increase in the frequencies of non-disjunctional (for the sex-chromosomes) spermatids over the control level. However, the frequencies of diploid spermatids increased linearly over the range of doses tested, but the slopes for the different sampling times were different. The cell stages most sensitive to the induction of diploid spermatids were sampled on days 4 and 9 after irradiation (day 4: primary spermatocytes in late pachyteny, diakinesis or metaphase and possibly some secondary spermatocytes at the time of irradiation; day 9: leptotene, zygotene and early pachyteny at irradiation).

385. The lack of response to the induction of sex-chromosomal non-disjunction is puzzling. The animals used in this and in the earlier studies were derived from a colony initiated in 1974 with 2 pairs of animals and 3 young. No specific breeding scheme was used except that in every generation, breeding pairs were usually formed by taking into account factors such as fertility, fecundity and absence of cannibalism. It may be that increased inbreeding might have selected against presumed non-disjunction-prone individuals. Current studies with a new colony established from new wild animals which are under way, may help to throw light on this possibility.

6. Summary and conclusions

386. A number of cytogenetic and genetic methods are currently available to study spontaneously-arising and radiation-induced chromosome losses and gains in the germ cell stages of experimental mammalian species. Most of the recent data have come from the use of cytogenetic methods (e.g., chromosome analysis of early cleavage stages, embryos at 9–10 days of gestation, oocyte stages at metaphase II, etc.).

387. The spontaneous incidence of monosomies and trisomies in early embryonic stages of most mammalian species studied in this respect is quite low, being of the order of about 0.5 to 1%. This is in contrast to the situation obtained in humans.

388. When male mice are exposed to x irradiation (100 R), mated to unexposed females and the embryos derived from irradiated meiotic and spermatogonial stages are analysed for aneuploidy, there is no measurable increase in the frequency of either monosomic or trisomic embryos over the control level. One study with a lower exposure of 20 R provides some evidence for a possible increase in the total frequency of aneuploids (monosomy and trisomy) and for the possibility that non-disjunction may occur at meiosis I or II; in the same study, at the higher exposure of 200 R, there seems to be no further increase in aneuploidy.

389. The problem of whether small x-ray exposures to female mice (in the range from 2 to 16 R) will lead to an increase in the frequency of non-disjunctional gametes leading to monosomic and trisomic embryos has been investigated by a number of workers. The available data suggest that this effect could not be demonstrated in either young or aged female mice.

390. The results of a genetic study in the mouse have provided no convincing evidence for irradiation
causing an increase in the frequency of X-chromosomal non-disjunctional progeny in irradiated young or aged females.

391. Studies involving irradiation of female Chinese hamsters (young and old; 20-200 R range) and subsequent culturing of their oocytes in vitro have provided no clear-cut evidence for an increase in the frequency of aneuploid eggs; there was no demonstrable effect of maternal age either. Other experiments with the same species aimed at ascertaining the oocyte sensitivity to the X-ray induction of aneuploidy (irradiation of females 85 to 7 hours before ovulation; 10 different time periods) show that there may be an increase in the frequencies of hyper-haploid and hypo-haploid eggs after irradiation with 200 R; the increases for any individual stage, however, are not significant in view of the relatively small sizes of the samples. In oocytes sampled 11 days before ovulation (and which manifest a high sensitivity to the induction of chromatid structural aberrations) for which data at 50, 100 and 200 R are available, there are indications that there may be an increase in the frequency of hyper-haploid and hypo-haploid eggs, but it is by no means as striking as it is in the case of chromatid aberrations.

392. In the Northern field vole, Microtus oeconomus, contradictory results have been obtained on the radiation-induction of sex-chromosomal aneuploidy in male meiotic stages; earlier experiments provided positive evidence while the later ones showed no significant induction (dose of up to 2 Gy); however, there is good evidence for the induction of diplody and the cell stages sampled on days 4 and 9 after irradiation seem to be most sensitive in this respect.

D. POINT MUTATIONS

1. Specific-locus mutations in male mice: effects of $^3$H and $^{239}$Pu

393. In the 1977 report, the preliminary results of Cumming et al. [C36, C37] on the induction of specific locus mutations by tritiated water in the germ cells of male mice were presented. More complete results from this work have now become available [R31]. Briefly, (101 x C3H) F1 wild type male mice were injected intraperitoneally with tritiated water (1.85 or 2.78 $10^{-2}$ MBq per gram of body weight) and mated to females of the tester stock. The offspring were scored for presumed mutations at the seven loci and the presumed mutants were bred to establish allelism of the mutations and to determine the viability of the mutations in the homozygous condition. Both post-spermatogonial and spermatogonial stages were sampled. The weighted mean dose for the experiments with irradiated post-spermatogonial stages was calculated by estimating the accumulated dose for each succeeding day after HTO injection and weighting by the number of offspring conceived that day. For the experiments with irradiated spermatogonial stages, the weighting was by weekly, rather than daily, intervals. The data are presented in Table 27. These data permit an estimate of an induction rate of 4.4 $10^{-7}$/locus/$10^{-2}$ Gy for post-spermatogonial stages and of 1.5 $10^{-7}$/locus/$10^{-2}$ Gy for spermatogonia after correction for control rate (historical control: 28 mutations in 531 500 offspring).

394. An examination of the distribution of the mutations in the offspring (Table 28) shows the following features:

(a) For spermatogonial irradiation, the pattern is not significantly different from that which has been recorded for X- or gamma-irradiation where a low frequency has always been observed at the α and se loci;
(b) The mutations in the offspring from irradiation of post-spermatogonial stages appear to be more evenly distributed among the loci, a characteristic of those obtained in X-irradiation experiments involving these stages [R32];
(c) Although no deficiencies involving d and se loci simultaneously has been recorded in the present study (these are common after X-irradiation of post-spermatogonial stages [R32, R33]) there is other evidence for the association of some of the mutations with aberrations: three of the mutants were sterile and an α locus mutant listed as "untested" had a high frequency of sterility in her offspring;
(d) In the spermatogonial series, 10 out of the 18 tested mutants were viable in the homozygous condition and this proportion was 3 in 11 in the post-spermatogonial group.

Altogether, these data present no evidence for any striking disparity from the spectrum and properties of mutations recovered in X-irradiation experiments.

395. Russell et al. [R31] have estimated the RBE values for mutation induction by $^3$H relating the mutation rates observed in the present work with those recorded in earlier investigations with X- or gamma-irradiation. For post-spermatogonial stages, the results of experiments with 300 R of x rays (90 R min$^{-1}$) was chosen as the standard although the dose rate in the $^3$H study was much lower than the rate of 90 R min$^{-1}$. The justification for this is that the mutation rate in post-spermatogonial stages appears to be independent of dose rate or at least not markedly affected by it [R34]. The x-ray data (based on the progeny from the first two weeks of mating) yield a rate of 6.1 $10^{-7}$/locus/$10^{-2}$ Gy (25 mutations in 18 693) after correction for controls [R35]. (The reason for using these data is that in the $^3$H experiments 95% of the progeny were conceived in the first two weeks after injection.) The rate in the $^3$H experiments namely 4.4 $10^{-7}$/locus/$10^{-2}$ Gy divided by the rate cited above for x rays gives an RBE of 0.7 (after making a correction from R to Gy) for the induction of specific locus mutations in post-spermatogonial stages.

With the limited number of mutations scored in the $^3$H series, the above RBE values are not significantly different from 1.

396. For spermatogonial stages, as is well known, dose rate is very important [R34, R36]. In the $^3$H experiment, the dose rate is relatively high in the first few days after injection as compared to what it is later, but even in the first half day, it probably does not average much more than about 0.001 Gy per minute. Consequently, the data most suitable for comparison are those obtained with gamma rays at 0.8 R min$^{-1}$ and below. The recent calculations of Russell and Kelly [R77, R78] show that for all such data, the straight line of best fit is described by Y = (8.04 $10^{-6}$ ± 1.19 $10^{-6}$) + (7.34 $10^{-8}$ ± 0.83 $10^{-8}$)D in which Y is the mutation frequency per locus and D is the exposure in R. This result does not differ greatly from the regression formula Y = (8.34 $10^{-6}$ + 0.659 $10^{-6}$)D calculated by Searle [S69] who restricted his analysis to the data then available at exposure rates of 0.009 R per minute and lower. Dividing the rate obtained with $^3$H (1.5 $10^{-7}$) by the rate for gamma rays estimated by Russell and Kelly (with correction for the change from R to Gy), one obtains an RBE value of 2.
for $^3$H relative to chronic gamma ray exposures of spermatagonia. Because of the limited number of mutations in the sample, uncertainty as to the number of independent events involved when clustering occurs, and some uncertainty as to the true does, Russell et al. [R31] believe that it is unlikely that the RBE value of 2.0 differs significantly from 1. They, however, stress the point that it would seem prudent at this time to assume that the RBE for exposure of stem cells in the testis to tritiated water might be approximately 2" and that this value might be used in the context of risk evaluations.

397. In the 1977 report, a review was made of the available data on the induction of dominant lethals [L33, S53] and cytogenetic damage [S53, B44] in male mice by $^{259}$Pu. Experiments have been initiated in Oak Ridge [R37] to study the induction of specific locus mutations with this radioactive isotope. In this work (101 x C3H/F1) male mice were injected intravenously with 0.37 MBq/kg body weight (~ 0.925 MBq/mouse) of monomeric $^{259}$Pu citrate. Thirteen weeks after injection, the males were mated with untreated females of the tester strain and the offspring are being scored for mutations at the seven specific loci. Until now, 11 presumed mutations have been observed in 34,670 offspring. This is highly significantly above the control frequency.

2. Specific-locus mutations in female mice

398. In earlier work discussed in the 1977 report, Lyon and Phillips [L31] investigated the mutational response of maturing mouse oocytes to x-irradiation with a total dose of 2 Gy given in 20 fractions of 0.1 Gy each. The offspring conceived within the first seven weeks (and later) after the last irradiation were scored for mutations at the seven loci of the PT stock. Appropriate controls with 2 Gy given acutely were run. The results showed that the yields with the fractionation regimes were lower than that after acute irradiation. Based on those data and earlier fractionation data of Russell [R39] Lyon and Phillips concluded that "...it seems reasonable to suppose that in our work, the effect of each 0.1 Gy dose was low, and equal to 1/20 of the observed effect of twenty such doses ... it seems probable that the effects per unit dose of a 0.1 Gy dose is indeed less than that of 0.5 Gy and hence the dose-response relationship is highly curved. There is a suggestion in Russell's data that the mutation rate per unit dose is still increasing at dose levels of 200 R to 400 R, but this needs further work". The experiments of Lyon et al. [L32] considered below were designed to examine the mutational response at relatively higher doses.

399. Adult female mice were given doses of 2, 4, or 6 Gy of x-rays at 0.52 or 0.72 Gy min$^{-1}$ and mated immediately. Offspring conceived in the first seven days (i.e., using oocytes which were mature at the time of treatment) were scored for specific locus mutations at the seven loci. The earlier data from their work at 2 Gy (acute irradiation; conceptions up to 10 days after treatment) were added to the results of 2 Gy in the present work; the control data were taken from Russell [R38].

400. The results are presented in Table 29. Statistical analysis was carried out by fitting the data to a linear model ($Y = c + bD$), a linear-quadratic model ($Y = c + aD + bD^2$) and to a square-law model ($Y = c + bD^2$). With the linear model, the values of slope varied from (3.13 ± 0.50) $10^{-5}$ to (3.06 ± 0.50) $10^{-4}$ depending on the control frequency, with the departure from linearity being marginally significant at the 5% level. With the linear-quadratic model, the values of a varied from (0.42 ± 1.05) $10^{-4}$ to (0.23 ± 1.05) $10^{-3}$ and those of b from (6.22 ± 2.60) $10^{-9}$ to (6.47 ± 2.60) $10^{-9}$ again depending on the control frequency assumed. The P values (from tests of goodness of fit) were from 0.49 to 0.50, showing that the fit of the data to the model was good. It may be noted that the linear term is not significantly different from zero. With the last model, the b values ranged from (6.99 ± 1.15) $10^{-9}$ to (7.19 ± 1.15) $10^{-9}$ and the P (goodness of fit) values, from 0.71 to 0.77 showing that the fit to this model is slightly better than that to the previous one.

401. The authors point out that the good fit of the data to the linear-quadratic or to the square-law models does not necessarily imply that these relationships give a good representation of the biological phenomena involved; that the observations are also amenable to interpretations based on dose-dependence of repair processes, as has been suggested earlier by Russell (see [R38] for a recent discussion); and that they prefer the interpretation based on the repair hypothesis. Their reasoning for the last conclusion is discussed in the next section.

402. Other useful information that was obtained in the study of Lyon et al. [L31] pertain to the distribution of the mutations among the different loci, their viability pattern, the induction of dominant or X-linked visible mutations and those with irregular inheritance. The spectrum of mutations resembled that found by Russell et al. [R40] and others [S69] in mouse spermatagonia in that approximately 50% occurred at the S locus, whereas mutations at the a and g loci were rare. There were no simultaneous d-se mutations (presumed to arise as deletions) although Russell [R33] had found such deletions to be common after irradiation of oocytes. The proportion of those which was lethal in the homozygous condition was 78%, in good agreement with that obtained by Russell et al. [R40] for spermatagonia and nearly all the s and d mutations were homozogous lethal. The dominant and X-linked visible mutations included 2 viable repeated and 2 lethal splotch (Sp) alleles and a lethal W allele. In addition there was a new X-linked mutation (broad-headed; Bhd) and 2 further mutations caused a light coat colour and/or spotting. Six additional mutants with irregular inheritance occurred and these had tail kinks, light coats, behavioural abnormalities and small size either together or in various combinations.

403. Selby et al. [S70] studied the mutational response of the oocyte stage in female mice shortly before birth. These mice were given 300 R of either x-irradiation at high exposure rate (93 R min$^{-1}$) or 300 R of gamma-irradiation at 0.8 R per minute. At maturity, the irradiated animals were mated to males of the tester stock to score for mutations at the seven specific loci. In the acute x-ray series, 3 mutations were recovered among 16 194 F1 progeny; in the gamma-ray series, 1 mutation was found among 37 218 progeny. These results are therefore consistent with the existence of a marked dose-rate effect of the kind recorded for maturing oocytes and the possibility that the repair capacity of the oocytes in mice shortly before birth may not be different from that of maturing oocytes in adult female mice.
3. Nature of specific-locus mutations

(a) Dose-response kinetics and the nature of specific-locus mutations.

404. The 1977 report of the Committee considered in detail the arguments of Abrahamson and Wolff [24] which led these authors to conclude that:

(a) The dose-rate effect for low-LET irradiation found in specific-locus experiments with mouse spermatogonia and oocytes is a consequence of the fact that a large proportion of mutational events at high dose rates are two-track events;

(b) The relative contribution of one-track and two-track events to the mutational yield could be estimated (for oocytes, this was done from data on acute x-irradiation at 50, 200 and 400 R and for spermatogonia, the linear component estimated from the available low dose rate data was used to estimate the magnitude of the two-track component after acute x-irradiation);

(c) The coefficients for one- and two-track components so obtained can be used to calculate expected frequencies of mutations in the other experiments;

(d) As the magnitude of the one-track component for oocytes is substantial (and since the oocyte data are used to predict genetic risks for irradiated human females) there is reason to consider that the earlier hazard evaluation (which assumed that the risk to human females from low-LET irradiation of mature and maturing oocytes at low doses or at low dose rates is only one-twentieth of that at high doses and dose rates) is too low and to revise it upwards.

405. In their recent paper on x-ray stage sensitivity of mouse oocytes, Brewn and Payne [B32] have drawn attention to the line of reasoning pursued by Abrahamson and Wolff discussed in the preceding paragraphs, in the interpretation of the mouse oocyte results. Brewn and Payne compared the yields of cytologically-scored chromosome deletions (observed in their study of oocytes irradiated in the intact animals and cultured in vitro with subsequent processing for chromosome aberration analysis) and specific-locus mutations (in Russell's studies). They found that, when appropriate corrections are made for changes in oocyte sensitivity with time after irradiation, both the data on deletions and specific-locus mutations gave good fits to a power law model of the form \( Y = kD^n \) where the slope of the line (in a log-log plot) is a first approximation of the dose exponent, \( n \). The exponent \( n \) approximated a value of 1.7 when the data were plotted by individual stages of sensitivity, i.e., week 1 for deletions and mutations, weeks 2 to 4 for deletions and weeks 2 to 6 for mutations. The data pooled over the entire time span tested, gave a slope of 1.7 for deletions over the entire exposure range studied; for mutations this was true from 50 to 200 R but between 200 and 400 R, \( n \) was equal to 1.1 (for deletions too, if the analysis was restricted to week 1 data at the higher doses, the same phenomenon was observed, namely, a lower \( n \)).

406. On the basis of these analyses, Brewn and Payne stated "... the similarities in the variations in sensitivity of induction as a function of oocyte maturation and the dose-response characteristics, when this stage sensitivity is accounted for, of both structural chromosome aberrations and specific locus mutations lead us to conclude that they are produced by the same general mechanism following ionizing radiation... We suggest that this mechanism involves, principally, the interaction of two independently induced lesions when low-LET radiation is employed and consequently follows approximate D2 kinetics. This proposal does not require any more complicated hypotheses to explain the data and is in accord with other radiobiological theory... this would explain the very low mutation yields following chronic or multiple small fractionated exposures..."

407. However, ever since his first discovery of dose-rate effects in spermatogonia and oocytes, Russell [R38] has continued to favour the alternative hypothesis (on the basis of a number of further studies and a solid body of supporting data) that the mutations themselves are single-track phenomena and other track events are involved in damaging or saturating the repair processes at higher doses and dose rates; that the hypothesis that the dose-rate effects observed may be predominantly a consequence of repair of one-track mutational events also fits the data indicating that the dose response for low-LET radiations for induction of point mutations in mammalian cells is probably curvilinear (concave at high, and linear at low dose rates; and that the approach used by Abrahamson and Wolff is not a reliable one for estimating hazards in man.

408. It may be recalled from subsection 2 that Lyon et al. [L32] consider that their oocyte specific-locus data are biologically more consistent with an interpretation based on repair mechanisms. Their arguments are that W.L. Russell [R38, R41] had shown that in young females, mutation rate varied with time after treatment. Weeks 2–6 were more sensitive than week 1, and from week 7 onwards, no mutagenic effects were obtained. The increased sensitivity of weeks 2–6 does not appear to occur at all doses. A similar variation with time after treatment is found for chromosome aberrations [B45, B46, C34] and dominant lethals [C34]; the frequency rises with time until stability sets in, and, as with gene mutations, the amount of increase is dependent on radiation dose [C34]. Further there is another factor which had been shown to affect sensitivity of oocytes to mutagenesis, namely, the age of females at irradiation; Russell [R36, R41] found that old females gave a higher mutation rate than young females, particularly in second litters. Females irradiated on the day of birth or as 17.5 day old foetuses however gave a lower mutation rate than those irradiated when adult, but the mutations occurred at later (≥7 weeks) intervals. Lastly, it is thus easy to see that all these different responses cannot be easily explained in terms of radiation hit kinetics.

409. Lyon et al. [L32] believe that the assumption of differing activities of compensating error-free and error-prone repair systems in oocytes such as those postulated to exist in microorganisms (see [K22] for a recent review) would provide ample ground for explaining the observed variations in mutability. First, the combination of sensitivity to cell killing and resistance to mutagenesis shown by small oocytes resting in small follicles (up to stage 3a [P27]) and sampled later than seven weeks [O9] is consistent with the absence of error-prone repair. One may thus postulate that small oocytes are characterized by weak error-free and absent error-prone repair. Any potentially mutagenic insult would lead to death of these cells; such an absence of error-prone repair would protect the germ cells from excessive mutagenesis at the expense of increasing cell death. Second, in growing follicles sampled in weeks 2-6, repair enzymes (including error-prone systems) would be among the proteins becoming functional in the developing oocyte.
The finding of Pedersen and Mangia [P28] that UDS in response to UV treatment was higher in growing than in resting oocytes is in line with this reasoning. The ability of the oocytes to survive mutagenic insults would increase, but at the expense of an increase in mutagenic response. Finally, in the mature follicle, in which UDS is high [M46], error-free repair would become stronger so that resistance to both cell killing and mutagenesis would improve.

410. Lyon et al. [L32] suggest that the above model involving varying levels of error-free and inducible levels of error-free and error-prone repair, although admittedly speculative, would fit the data on mutagenesis in mouse oocytes well. It would also be possible to explain variation among species, or among strains within a species, in a similar way. The details of these phenomena however, remain to be elucidated.

(b) Further data from the analysis of the albino locus "c" region

411. In the 1977 report, some data of L.B. Russell et al. on the c (albino) locus mutations were presented. The study is now completed [R42, R43, R44, R79]. Altogether, 119 presumed mutations involving the c locus were recovered in more than two decades of work by W.L. Russell et al. on the radiation-induction of specific locus mutations in various germ cell stages and with different kinds of irradiations and regimes and these constituted the material for the study of L.B. Russell et al. Of these 107 were fully tested for allelism and for viability in the homozygous condition; of the remaining 12, one was tested for allelism only, four died before they were old enough to reproduce and 7 were not mated.

412. The 107 tested mutations may be subdivided on the basis of

(a) Viability in the homozygous condition (viable, subvital and neonatally or prenatally lethal, designated with v, s, and l, respectively, as part of their superscript);

(b) Whether with respect to pigment phenotype in various combinations, they mimicked the "albino" allele (mutants designated with a as part of their superscript) or were intermediate between c and C (designated with ch or c as part of their superscript). Fifty five of the mutations were viable and albino (c\(^a\)), 13 were viable and of various intermediate pigment types (c\(^a\)), 4 were subvital (c\(^a\) and c\(^s\)), 7 neonatally lethal albino (c\(^a\)) and 33 prenatally lethal albino (c\(^al\)).

413. All the prenatally lethal and at least one of the neonatally lethal c locus mutations (c\(^al\)) are probably deficiencies. Since the absence of the locus mimics albino in phenotype, the intermediates are assumed to result from intragenic changes. There is evidence that the class of viable albino mutants are either intragenic changes or very small deficiencies.

414. The distribution of the major classes of the mutations are given in Table 30 from which it can be seen that:

(a) Among the x- or gamma-ray-induced mutations, 34 of the 51 induced in spermatogonia (67%) are homozygous viable; this frequency is slightly lower for neutron-induced c locus mutations (9 out of 15);

(b) The total number of mutations that were available from other cell stages is relatively small;

(c) The proportion of the mutations that are lethal homozygously is of the order of about 30% for those recovered after spermatogonial irradiation (x- or gamma-ray irradiation); for other cell stages, the overall proportion of lethals is almost twice as high (58%; 14/24);

(d) The proportion of intermediate types among the 34 non-lethal, non-mosaic c locus mutations recovered from irradiated spermatogonia (x- or gamma-ray irradiation) is 26%, which is nearly the same as that among spontaneous mutations (28%); none of the 9 whole-body non-lethals derived from the neutron-irradiated spermatogonial group was of the intermediate type. It would thus appear that low-LET irradiation of spermatogonia increases the absolute frequency of viable c locus mutations without changing the spectrum from that of spontaneous ones;

(e) The proportion of homozygous viables is highest for the spontaneous c locus mutations.

Not shown in Table 30 is the finding that there was no significant dose rate effect on the relative proportions of c locus mutations that were homozygous lethal. L.B. Russell et al. [R42] consider that these findings support the view that most of the c locus mutations induced in spermatogonia even by high dose rate x- or gamma-irradiations are of a type most likely to result from single-track events (c\(^e\)) since (c\(^e\) and c\(^a\) plus 16% presumed deficiencies not involving the closest marker) and that most of the reduction in mutation frequency at low dose rates is not due to a change in relative proportion of two-track and one-track ionizing events.

415. Turning now to fractional c locus mutations, 16 of them were recovered among the total of 119 [R43]. These 16 mutations were distributed evenly among controls and irradiated groups, suggesting that the fractions observed are all presumably spontaneous in origin. (At the c locus, the bulk of spontaneous mutations have been fractional.) The overall progeny ratios from the fractional mutants indicated that the mutations could have occurred in one strand of the gamete DNA, in a daughter chromatid derived from pronuclear DNA synthesis, or in one of the first two blastomeres prior to replication.

416. The stage at which homozygotes die was determined for 28 c locus mutations of which 26 had earlier been found to be probably prenatally lethal [R45]. Uterine dissection experiments involving 796 dissected females and 7615 corpora lutea [R44] indicate that the prenatally lethal mutants die either around early preimplantation (13 mutants) or around the time of implantation (13 mutants). None of the c locus mutations kills between the latter time and the time around birth, when the neonatal lethals (7 mutants) die.

4. Autosomal recessive lethals in male mice

417. The primary aim of the work of Lüning et al. [L18] described earlier in the section on dominant lethals was to assess the effectiveness of acute and chronic irradiation with 14.5 MeV neutrons in inducing autosomal recessive lethals in mouse spermatogonia. Adult CBA male mice were irradiated with 1.5 or 2.5 Gy (acute) or 2.5 Gy (chronic: about 7 x 10\(^3\) Gy h\(^{-1}\), 8 hours a day for 5 days a week through 11 weeks). The two important findings from this study are that:
(a) There is no measurable difference in the rate of induction of autosomal recessive lethals in mouse spermatogonia between neurons delivered acutely or chronically.
(b) The rate of induction is about $2 \times 10^{-4}/10^{-2}$ Gy/game, 14.5 MeV neutrons being about twice as effective as acute x rays.

5. Sex-linked recessive lethals in male mice and rats

418. In the 1977 report, the details of an X-chromosome inversion in X1H described by Evans and Phillips [E12] were given. This inversion covers about 85% of the physical length of the X chromosome and, therefore, covers slightly over 5% of the mouse genome. Lyon, Phillips and Fisher [LSO] have now reported on the use of this inversion to study the x-ray induction of X-linked recessive lethals in spermatogonia. Males were irradiated as young adults with a fractionated dose of $5 + 5$ Gy of x rays (0.9 Gy min$^{-1}$) with a 24 h interval. It was found that 2/536 irradiated and 0/529 control X chromosomes carried a confirmed lethal. These frequencies correspond to a rate of induction of $1.9 \times 10^{-6}$ per 10 Gy per X chromosome for single exposures (allowing for the enhancing effect of fractionation). The results are considered as fully consistent with previous experiments in which X-linked lethals were not detected and indicate the value of using inversions for this work and the need for large-scale experiments. The results are also consistent with those on the induction of autosomal recessive lethals although there were indications that some X-linked lethals may be eliminated in the heterozygous state.

419. Chambers and Chapman [C29] have recently reported on the rate of induction of sex-linked recessive lethals in rat spermatogonia. The total x-ray exposure was 450 R given in three fractions of 100, 150 and 200 R at 10, 12 and 14 weeks of age, respectively. The data used for estimating the rate of sex-linked recessive lethals were obtained by irradiating one litter at a time and expanding the number of litters with the earlier estimate of $1.6 \times 10^{-4}/X$ chromosome/R and in good agreement with the estimates of 1.6 $10^{-4}/X$ chromosome/R by Taylor and Chapman [T13].

6. Autosomal recessive lethals in female mice

420. Lüning and Eiche [L34] have collected data on the induction of autosomal recessive lethals in maturing oocytes of mice of their CBA strain. The procedures and test schemes were essentially those used earlier in connection with work on the determination of radiation-induced autosomal recessive lethals in spermatogonia [L36, L37]. The x-ray exposure was 250 R to sexually mature females; the irradiated females were mated to males immediately after irradiation and were allowed to produce one or two litters conceived during the first six weeks following irradiation. The data are consistent with a rate of induction of $1.3 \times 10^{-4}/$ R/game by one method of calculation [H15B] or of 0.8 to 1.2 $10^{-4}/$ R/game by another method [L35]. In any case it is clear that these estimates are about the same as that arrived at for spermatogonia [L47].

421. In other experiments with irradiation of female foetuses [L34] x-irradiation of pregnant females with a fractionated exposure: 25 R d$^{-1}$ for four days from day 10 to day 13 of pregnancy plus 50 R d$^{-1}$ for a further four days from day 15 through day 18; total accumu-

lated exposure: 300 R) it was found that the mortality in the F2 crosses was no higher than in controls. This suggests that the germ cells of female foetuses may not be more sensitive than maturing oocytes of adult females and that the risk from foetal exposure is no higher than that from adult exposures.

422. Rönnebäck and Sheridan [R46] irradiated female CBA mice chronically with $^{137}$Cs gamma rays in utero during either of two periods, the 10th to 14th days of gestation or 14th to 18th days of gestation. The doses administered were 0.34 Gy/generation in the first group and 1.6 Gy/generation in the second group. The dose rates were 3 $10^{-3}$ Gy h$^{-1}$ in the first and 1.7 $10^{-2}$ Gy h$^{-1}$ in the second group. The irradiation exposures were administered through nine generations and at the end, the female progeny were tested for the induction of autosomal recessive lethals. The data obtained are consistent with rates of induction of $1.5 \times 10^{-4}/10^{-2}$ Gy/game (in the group that received 0.34 Gy/generation during days 10-14) and 0.3 $10^{-4}/10^{-2}$ Gy/game (in the group that received 1.6 Gy per generation during days 14-18. The rate of 1.5 $10^{-4}$ is in accordance with that reported by Rönnebäck [R47], i.e., 6.3 $10^{-4}/10^{-2}$ Gy/game (with a range of 0.7 to 11.8 $10^{-4}$; 95% confidence limits) after a single chronic gamma radiation exposure of female foetuses (1.6 Gy) during the 10th to 14th days of gestation.

7. Biochemical mutations detected by electrophoresis

423. In the 1977 report, data on the induction of mutations in mouse spermatogonia, using nine electrophoretically detectable markers [M47] and haemoglobin loci [R48] were discussed. Narayanan et al. [N14] has now obtained some data in a spermatogonial irradiation experiment in which biochemical mutations at 20 loci were studied. Adult C3H males were irradiated with $^{137}$Cs gamma rays (100 + 500 R, separated by a 24 h interval) and mated to females of the 101 strain. Liver biopsy was performed on the offspring in order to screen for biochemical variants. Liver extracts were then submitted to isoelectric focusing on polyacrylamide gels, an electrophoretic method that is capable of separating proteins from one another differing in as little as 0.01 pH unit in their isoelectric points. Among 2100 F1 animals as far screened, three isoenzyme variants (two esterase and one lactate dehydrogenase variants) were recovered. In the controls, two esterase variants were found among 12,800 animals. A puzzling feature of the variants recovered in this work is that they were not transmitted beyond the F1.

424. In another study, Pretsch [P40] used a thin-layer chromatographic method to detect the induction of mutations causing hyperuricoacidemias. Adult C3H males or (101 X C3H)F1 males were given 100 + 500 R of $^{137}$Cs gamma-irradiation (24 h interval) and mated to females of the 101 or T strain, respectively. Both pre- and post-meiotic stages were sampled. The blood from the F1 animals was used for chromatography. No mutants, however, could be detected in a sample of 5786 animals.

8. Dominant mutations

425. In the 1977 report, the unpublished results of Selby and Selby on the induction of mutations causing dominant effects in the skeleton of the mouse were described. These data and discussions about their
relevance for hazard evaluations have now been published [E16, E17, E18, R49, S71, S72, S73, S74, S75, S117]. In this work, adult male mice received a fractionated gamma-irradiation exposure (100 + 500 R, separated by 24 h; 60 R min⁻¹) and were mated with untreated females. The F₁ sons conceived during the post-sterile period were processed for examination of their skeletons after they were allowed to produce progeny. Thirty-seven of the 2046 F₁ males were judged to have dominant mutations that caused one or more rare skeletal abnormalities; 31 of these were shown to be mutants by breeding tests and the remaining 6, having no progeny, were counted as mutants based only on criteria supported by the data.

426. In breeding tests, the authors found that the dominant mutations affecting the skeleton showed variable expressivity and incomplete penetrance for many or all of the effects that they caused. A number of them severely affected viability. With the experimental procedure used, both incomplete penetrance and decreased viability would have caused an underestimate of the mutation rate. Thus, some F₁s actually having a mutation might not show it because of low penetrance, some mutant F₁s having defective skeletons might not be classified as mutants if penetrance were too low to permit proof of transmissibility, and mutants that died before examination would not be counted. Note, however, that the first of these three cases does not lead to any underestimation of first generation genetic damage.

427. In order to know if some of the skeletal mutations might actually be gross chromosomal changes such as reciprocal translocations or aneuploids, cytogenetic examination of the testes of mutation-bearing males was carried out on 9 of the skeletal mutants (6 of which were proved mutants and 3 of which were counted as mutants on the basis of presumed-mutation criteria), 2 non-skeletal mutants and one control F₁. These were chosen for cytogenetic examination because they were either sterile or had partial sterility. Mutant numbers 1268, 2490 and 2691 were all found to be balanced reciprocal translocation heterozygotes: a heterozygote for a non-skeletal mutation (a belly-spot; mutant number 2971) which was partially sterile was also found to be a translocation carrier [S72]. Subsequent breeding tests involving mutant numbers 1268, 2691 and another mutant (number 1163) revealed that every mouse that had the reciprocal translocation had the skeletal defects and conversely, every one that did not have the translocation did not have skeletal defects. It thus appears that at least for these mutations, the association between the presence of a balanced reciprocal translocation and skeletal defects is strong and suggests that some of the balanced reciprocal translocations are in fact associated with dominant phenotypic effects.

428. In further experiments [S75] Selby tested eight other skeletal mutations for effects in homozygous condition. It was found that all of them were lethal as homozygotes; for seven of them, death occurred after implantation and before birth, and for the remaining one, 10 days after birth. Thus, at least in this limited sample, the majority of mutations having dominant effects on the skeleton in heterozygous carriers also act as recessive lethals. The follow-up experiment in which males known to be heterozygous for a skeletal mutation (mutant number 1629) were mated to normal (+/+ +) females, showed that the intra-uterine death rate was higher than what would have been expected on the basis that the mutation had no heterozygous effects on dominant lethality. From these results and other considerations, Selby has concluded that recessive lethals often have dominant harmful effects.

429. In addition to the skeletal system discussed above, there is one other system that has already been shown to be useful in studies on the induction of dominant mutations. This concerns dominant mutations causing cataracts in the eye of the mouse. The fact that cataracts can be caused by dominant mutations in mammals (including humans) is known [E19, M16, P30, W21, W27]. The experiments of Ehling and colleagues [E13, E20, E21, E24, K23, K24, K42] have validated the usefulness of the cataract system for both radiation and chemical mutagenesis studies. The paper of Ehling [E24] is the most recent on this subject. The radiation work of these authors consists of three experiments, one involving a total exposure of 910 R given in two equal fractions separated by a 24 h interval and the other two involving single exposures of 534 R and 600 R, respectively. In all experiments, adult (101 x C3H)F₁ male mice were gamma-irradiated at a rate of 53–55 R per minute and mated to females of the seven-locus tester stock (to score, in the same experiment, for specific locus mutations) and the eyes of the F₁ progeny at about 3 weeks of age were examined biomicroscopically with the aid of a slit lamp to detect lens opacities. All the presumed mutants were progeny-tested to confirm the genetic nature of the lens opacity phenotype. Specific locus mutations were scored using standard procedures.

430. The data are summarized in Table 31a which shows that the mutation rate for post-spermatogonial stages is higher than for spermatogonia and that exposure fractionation has an augmenting effect on mutation frequency. These responses have long been known from specific locus work and the present work has demonstrated that similar responses are obtained with cataract mutations. The mutation rate estimate that can be derived from these data are: 0.45–0.55 mutation per 10⁶ gametes per R for single high dose rate gamma ray exposures and 1.26 mutations per 10⁶ gametes per R for the particular fractionation regime used.

431. Small lens opacities frequently occurred in the offspring of the experimental as well as control groups (opacities of the anterior embryonic suture, opacities of the posterior embryonic suture, small white patches differing in form and location and remnants of the pupillary membrane). The total frequency of offspring with any posterior lens abnormality was 10% in the control group and 11% in the experimental group. Breeding tests of approximately 300 carriers provided the evidence that these small lens abnormalities are not caused by single dominant genes [K42]. These abnormalities were therefore excluded from the data presented in Table 31a.

432. The properties of the transmissible radiation-induced dominant cataract mutations are given in Table 31b. It can be seen that apart from two mutants which were sterile, the remainder were fertile. The penetrance and expressivity properties of these mutants are similar to those affecting the skeleton.

433. When one compares the overall frequencies of induced cataract and specific locus mutations (columns 4 and 7, respectively, in Table 31a), it is clear that the recessives outnumber the dominants by a factor of
about 2.5 for spermatogonial and 2.7 for post-spermatogonial irradiation. In humans, 20 well-established dominant cataract mutations are currently known [M16]. If the number of loci at which dominant cataract mutations can arise in the mouse is the same as in humans, then one is scoring for events at 20 loci as far as dominant cataract mutations are concerned, compared to events at 7 loci scored in the specific locus experiments (a factor of 3). Thus, on a per locus basis, radiation induces approximately the same (i.e. 2.5 x 2.7 x 3 ≈ 8) more recessive visible mutations than cataract mutations [E24].

9. Histocompatibility mutations

434. In the 1977 report, the studies of Kohn and colleagues on the induction of histocompatibility (H) mutations in mouse spermatogonia were described. As is well known, the H-system in mammals comprises a group of co-dominant genes located throughout the genome, and their action (production of cell membrane alloantigens) determines the acceptance or rejection of dermal grafts. For operational reasons, the H-loci in mice are divided into two classes distinguished from one another in the F1 hybrids of the B6 and C lines that are used. The class-I loci, at least 30 in number [B73], have different alleles in the parental lines and are therefore heterozygous in the F1 hybrid. The class-II loci have similar alleles in the parental lines and are therefore homozygous in the hybrid; the number of class-II loci is unknown, but a conservative estimate is of the order of about 50 loci [G42].

435. The procedure to detect new mutations is to exchange tail skin grafts orthotopically between F1 hybrid mice (derived from mutagenized germ cells) from the two strains mentioned above and classify the progeny as resulting from “gain” type mutations (appearance of a new antigenic specificity, i.e., grafts donated by the putative mutant rejected) or “loss” type mutations (loss of specificity i.e., grafts placed on the putative mutant rejected) or “gain-loss” type mutations (one specificity replaced by another). Class-I mutations are distinguished from class-II by their “loss” or “gain-and-loss” phenotypes for loci on autosomes and X chromosomes; class-II mutations could produce only “gains” unless the Y chromosome is involved (see [K38] for detailed description of the methods).

436. The earlier results of Kohn et al. [K38, K39] showed that there was no evidence for the recovery of x-ray induced histocompatibility mutations (spermatogonial irradiations). This result was attributed by the authors, not to the failure of the system to detect mutations, but rather to the very low mutability of these loci (relative to the seven or six specific loci that have been used in radiation studies with mice). They surmised that this could happen if the x-ray induced H-mutational lesions failed to result in viable progeny (see also [K40]).

437. In a further study, Dunn and Kohn [D23] tested for the induction of H-mutations in post-mitotic male germ cell stages. The doses used were 3.5, 6.5 or 3.5 + 3.0 Gy with a 24-h interval. Altogether 8 H-mutations were found in nearly 3000 progeny (including 1101 control progeny). Two of them were clusters and therefore could not have occurred in sperm; one class-I mutation was not relevant because it occurred in the B6 genome of the unirradiated mother. Of the relevant five, two were class-I losses (1 from the 6.5 Gy group; 1/565; the other was in the 3.5 + 3.0 Gy group; 1/514) and the remaining three were class-II mutations of the “gain” type: one on the control (1/1101), one in the 3.5 Gy group (1/809) and one in the 3.5 + 3.0 Gy group (1/514).

438. A comparison of these results with those for specific locus mutations in post-meiotic male germ cells and with spermatogonial mutation rate for the H-loci clearly indicates that in the first comparison, the rate for the class-I H-loci is only about 1/9 of the specific-locus rate; in the second comparison, the two rates do not significantly differ from one another. At face value, the spermatogonial rate for the H-loci is only about 1/60 of that for specific-locus mutations. In any case, the very low radiation-induced mutability appears to be a general property of these loci, one which does not depend on germ cell stage.

10. Induction of congenital anomalies and tumours by irradiation of mouse germ cells

439. Recently, Nomura [N23] published a paper in which he demonstrated that congenital anomalies and tumours could be detected in the progeny of irradiated mice and has thus identified a potentially useful model system for the study of these effects following irradiation of germ cells. Mature male or female mice (63–65 days old) were x-irradiated with doses in the range from 0.36 to 5.0 Gy and mated to unirradiated animals. Living foetuses on day 19 and offspring surviving more than 7 days after birth were examined for the incidence of congenital anomalies. (The germ cell stages sampled are not specified.) The results are summarized in Table 32.

440. It can be seen that there is a significant induction of congenital anomalies following germ cell irradiation and that the frequencies decrease (relative to those obtained in living foetuses) in live-born progeny. This is due to the fact that some anomalies (cleft palate, exencephalus etc.) are lethal shortly after birth. Since the data are presented as frequencies pooled over all radiation doses employed, no conclusions can be made with respect to whether there are dose-effect relationships or whether certain specific kinds of anomalies predominate at lower versus higher doses.

441. In the other part of the study dealing with tumours, the progeny of exposed parents (same doses as in the congenital anomalies part of the study) derived from irradiated spermatogonia, spermatids, spermatogonia and maturing oocytes were screened for the presence of tumours in their internal organs. The total yields of tumours in the offspring for all male germ cell stages sampled were significantly higher than in controls. Tumour incidence and lung tumour frequency were slightly higher when x-irradiation was administered 8–14 days before mating than at other intervals. Exposure of oocytes in early follicular stages (8–21 days before ovulation) induced significant yields of tumours while mature oocytes (1–7 days before ovulation) were resistant to 2.16 Gy of irradiation. When the dose was fractionated (2 equal fractions separated by 24 hours), increased yields (relative to single doses) were found for irradiated spermatogonia and mature oocytes, but not for spermatogonia and spermatids.

442. Kirk and Lyon [K43] have now confirmed Nomura’s findings with respect to the induction of
congenital anomalies in the offspring of exposed female mice. These were irradiated with 1.08 to 5.04 Gy acute x irradiation and mated at intervals of 1-7, 8-14, 15-21 and 22-28 days, with uterine examination in late pregnancy to detect early foetal deaths (dominant lethality) and late malformations. At each weekly interval, the incidence of abnormalities (with dwarfism and exencephaly as the commonest types) tended to rise with increasing dose, and at any given dose the incidence tended to rise with time after irradiation. Changes in incidence of dominant lethals and of abnormal foetuses paralleled each other closely, with highest incidences in week 3 (59 ± 5%) for dominant lethals and (12.5 ± 3.1%) for abnormal foetuses after 5.04 Gy). This increased radiosensitivity of less mature oocytes is similar to that reported previously for other genetic effects.

11. Summary and conclusions

443. Subsequent to the publication of the 1977 report, more data have been obtained on the induction of specific-locus mutations in the germ cell stages of male mice by tritium (given as tritiated water by i.p. injections). The data permit an estimate of induction rate of 4.4 × 10⁻⁷/locus/10⁻³ Gy of beta-irradiation for post-spermatogonial stages and of 1.5 × 10⁻⁷/locus/10⁻³ Gy for spermatogonia. The RBE estimate (relative to high dose rate x- or gamma-irradiation) for post-meiotic germ cell is not significantly different from unity. For spermatogonia, the RBE value (relative to chronic gamma rays) is about 2.2 although this value may not be significantly different from 1.

444. An examination of the distribution of the mutants among the seven loci reveals that the pattern is not significantly different from that recorded for x- or gamma-irradiation for spermatogonial mutants: the mutants obtained from irradiation of post-meiotic germ cells are more evenly distributed among the loci, a characteristic of those obtained in x-irradiation experiments involving these stages.

445. The preliminary results obtained in studies with ³²³Pu show that this radioactive isotope is capable of inducing specific-locus mutations in spermatogonia.

446. Further data have been obtained by Lyon and colleagues on the x-ray induction of specific locus mutations in maturing mouse oocytes at dose levels of 2, 4 and 6 Gy. When these data were analysed statistically, it was found that they fitted all three models tried (linear, linear-quadratic, power-law model) although the linear-quadratic and power-law models gave slightly better fits. It has been pointed out that the good fit of the data to the linear-quadratic and power-law models does not necessarily imply that these relationships adequately reflect the biological phenomena involved. Lyon and colleagues have expanded on the repair model originally proposed by W.L. Russell to explain these results.

447. The frequencies of specific locus mutations recovered from irradiation of oocyte stages in female mice shortly before birth at a low exposure rate of 0.8 R min⁻¹ (gamma rays: 300 R) were significantly below those obtained after the same exposure given at a high exposure rate (93 R min⁻¹); this observation suggests that the repair capacity in these oocyte stages may not be very different from that in oocytes of adult female mice.

448. Further data on the nature of mutations induced by ionizing radiation in mouse germ cells have been published pertaining to the analysis of the mutations recovered at the c (albino) locus in different radiation experiments. These show that for spermatogonial irradiation, nearly two-thirds of the x- or gamma-ray induced mutations are homozygous viable. The proportion is slightly lower for neutron-induced c locus mutations. Most of the lethal mutations are probably deficiencies. Since the absence of the locus mimics albino phenotype, the intermediate types (with respect to pigment phenotype) which were recovered are assumed to result from intragenic changes. The proportion of intermediate types among the 34 non-lethal, non-mosaic c locus mutations recovered from irradiated spermatogonia (x- or gamma-irradiation) is nearly the same as that among spontaneous mutations (20% and 28%, respectively). The viable albinos (as well as the intermediate types) are concluded to result from one-track events. There was no dose rate effect in the relative proportion of c-locus mutations that were lethal.

449. The 14.5 MeV neutrons are about twice as effective as acute x rays in inducing autosomal recessive lethals in mouse spermatogonia. At dose levels of 1.5 or 2 Gy, there is no dose rate effect for the induction of recessive lethals.

450. The rate of induction of autosomal recessive lethals in mature mouse oocytes after x-irradiation (high dose rate) is about the same as that recorded for spermatogonia; the germ cells in female foetuses (fractionated irradiation from day 10 through day 18 of pregnancy) do not appear to be more sensitive than maturing oocytes in adults.

451. Further studies with the skeletal mutations in mice have shown that some of the skeletal mutations may be associated with balanced reciprocal translocations; put in another way, some of the balanced reciprocal translocations are associated with dominant phenotypic effects. Other data on skeletal mutations show that all the eight tested were lethal in the homozygous condition.

452. New data have become available from studies on the induction of another kind of dominant mutation, namely, those that cause cataracts in the eye of the mouse. The induced cataract mutations are qualitatively similar to those affecting the skeleton in terms of dominance, penetrance and expressivity.

453. New data on the induction of histocompatibility mutations in post-meiotic male germ cell stages of the mouse show that the rate of induction by x-irradiation is much lower than that for recessive specific locus mutations; these data and those on irradiation of spermatogonial stages (in these stages too, the rate is much lower than that for specific locus mutations) confirm the view that the H-loci in general are much less mutable than the specific loci at which mutation induction has been extensively studied.

454. The studies of Nomura have shown that congenital anomalies and tumours can be recovered in the progeny of irradiated male and female mice. It thus appears that the mouse can serve as a useful model for the study of these kinds of effects following irradiation of germ cells.
E. GENETIC EFFECTS OF INTERNAL EMITTERS

455. In earlier reports of the Committee as well as in this Annex, data on the induction of genetic (and some other biological) effects by incorporated radioactive isotopes have been dealt with. The purpose of this section is to present a broad synthesis of all this information (for a recent review, see [S76]).

456. Studies on the genetic effects of incorporated radioactive isotopes present some special problems which are not encountered in other branches of radiation genetics and which, in many ways, are very similar to those encountered in chemical mutagenesis. Thus, for working with radioactive isotopes it is important to know the extent to which the nuclide actually reaches the gonads and how long it remains there, as well as its actual relationship to the germ cells themselves. Except with internal gamma-emitters, track-lengths are usually too short to allow the germ cells to be affected by any extra-gonadal material. Sometimes, the nuclide can become incorporated into the hereditary material itself and then its exact position and what chemical changes follow its decay may affect the likelihood of a mutational event after the transmutational one. These and other complex phenomena associated with the disintegration of a radioactive material mean that its actual effectiveness in mutation induction will not be determined solely by the characteristics of the emitted radiation.

457. Table 33 gives details about some internally deposited radionuclides of special genetic interest. Three of the $\beta$-emitters included are of obvious interest because they can be incorporated into DNA. The penetrating gamma radiation from $^{137}$Cs will reach the gonads from anywhere in the body, but $\beta$-radiation from gonadally deposited material is of importance as well. The three alpha emitters are known to reach soft tissues (including gonads) after being taken into the body and to be retained there for long periods [I4, R51]. Environmental contamination from these three radionuclides arises in various nuclear operations, such as mining and milling and fuel processing, while it has been estimated that thermonuclear reactors would produce 105 times as much tritium per megawatt as fission reactors [S77].

458. A summary of some of the main results obtained with different radioactive isotopes studied in mammals is given in Table 34. It is clear that the work done so far in this area is relatively meagre and diffuse but it has already exposed some intriguing aspects of biological response. For instance, there is the apparent high cytogenetic sensitivity of rat spermatozoa to low doses of certain $\beta$-emitters [B47], the later intra-uterine deaths after $^{239}$Pu alpha exposures which continued into the next generation [L33] and the declining translocation yield with prolonged exposures of male mice to $^{239}$Pu despite high retention [B44, G23]. All these and other problems need more detailed studies.

F. OTHER RELEVANT DATA

1. Biological effects in mice and rabbits kept in an area of high natural radioactivity

459. Léonard et al. [L21] have reported on their observations of biological effects in male mice and rabbits artificially kept in area near Lodève in South-western France. Exceptionally high concentrations of uranium (of the order of 1% and sometimes up to 7-8%) are found at certain sites, giving rise to a very high natural radioactivity. Correspondingly high concentrations of radon occur in the atmosphere. At a site where the dose rate amounts to about 8 $10^{-5}$ Gy h$^{-1}$, a rabbit hut was built and cages with laboratory rabbits were placed on its floor. Gamma-ray dose was determined by placing individual lithium fluoride dosimeters around the neck of each rabbit. Air samples inside the hut were taken in spring and autumn for the determination of $^{222}$Rn. The rabbits were kept in the hut for a period of 12 months, and blood samples were taken at 4-month intervals to study chromosome aberrations in lymphocytes. Control animals were kept in a nearby site with very much lower level background radiation, as well as in the laboratory.

460. Male mice of the BALB/c strain were also placed in the hut during summer; they could not be maintained for longer periods owing to their sensitivity to climatic variations. Control mice were kept in the same hut as control rabbits. The effects studied included those on fertility and the induction of heritable chromosome aberrations.

461. The external gamma radiation dose received by individual rabbits varied between 0.13 and 0.26 Gy after 120 days, 0.15 to 0.53 Gy after 240 days and 0.21 to 0.71 Gy after 360 days. The concentration of radon in the rabbit hut was $\sim 5$ kBq l$^{-1}$ in autumn and $\sim 90$ Bq l$^{-1}$ in the spring. It was estimated that at these radon concentrations, an annual dose of about 5-10 Gy would have been delivered to the bronchial region, the doses to other tissues being much smaller (e.g., gonads: annual dose of about 8 $10^{-5}$ to 1 $10^{-2}$ Gy). In mice, the total accumulated gamma dose was estimated to be 0.14 Gy for the 3-month period.

462. The cytogenetic observations on rabbit lymphocytes showed that in those cells sampled prior to radiation exposure, the only aberrations noted were chromatid and chromosome gaps and chromatid fragments and this was true of controls as well. After the exposure, in addition to these aberrations, dicentrics were found; the total frequency of abnormal cells rose to 1.90% by four months, 2.63% by eight months and 3.9% by one year (the control value was one-third of the last mentioned figure after one year). In the mice in which both the irradiated males and their F1 sons were studied cytologically for the presence of translocations or other aberrations in their spermatocytes, none were found.

463. In a subsequent paper, Léonard et al. [L22] extended these observations to a further eight months for rabbits and also conducted further studies with mice. In rabbits, the lymphocytes of which were sampled at 16 months, difficulties were encountered to stimulate the lymphocytes to divide and the aberration frequencies became very variable between the different animals. In general these frequencies did not exceed the level observed after 12 months and in fact showed a reduction. By 20 months, there were practically no aberrations (1000 cells from 7 animals). Male mice kept in the radiation area for three months showed slight increases in litter size, the mean number of offspring sired and in the mean number of offspring weaned over a 6-month period (estimated external dose: 0.15 Gy). Female mice, whose biological response was also studied, however, showed slight reduction in the mean number of litters, mean number of offspring sired and in the mean number of offspring weaned. Model experi-
ments with radon exposure of laboratory rabbits under controlled conditions (to concentrations that were roughly five times the total exposure of the rabbits maintained in Lodève for eight months) showed no chromosome aberrations, suggesting that the chromosome aberrations observed in the earlier study were probably not due to radon exposure but essentially to gamma irradiation.

2. Further data on the relationship between chromosome arm number and relative radiosensitivity in different mammalian species

464. It may be recalled that in the 1977 report, the "arm number hypothesis" proposed by Brewen et al. [B39] and the data collected by other authors to further examine the validity of the hypothesis were discussed (see also [S64]). It was concluded that the arm number relationship (i.e., the linear relationship between the yield of dicentrics and the effective chromosome arm number after low-LET irradiation) was not adequately documented to be used at present to predict from lymphocyte data of one species the expected frequencies of dicentrics in lymphocytes of another, or to estimate the frequencies of reciprocal translocations in spermatocytes of one species from those of another. Furthermore, the two-fold higher sensitivity of human lymphocytes to the induction of dicentrics (relative to the mouse) could not be confirmed.

465. Subsequent to the publication of the 1977 report, some new data have become available. Considering first the comparison of the sensitivities of human and mouse lymphocytes to the induction of dicentrics, the earlier data of Brewen et al. [B39] had been collected using culture times of 60–63 h for the mouse and 54 h for human lymphocytes. The work of de Boer et al. [B40] demonstrated that, in the above investigation, the frequencies of dicentrics scored in mouse lymphocytes were based on cells that were both in the first and second division after irradiation; when a 36 h culture time was used (scoring only first division cells), the frequencies of dicentrics were nearly the same as those in human lymphocytes and this was true after 1 Gy as well as after 2 Gy of x rays, i.e., there was no relationship between chromosome arm number and relative radiosensitivity to the induction of dicentrics (mouse: 40 chromosome arms; man: 81 chromosome arms).

466. Preston and Brewen [P26] re-examined the above problem by studying the frequencies of induced dicentrics using different fixation times (42 and 48 h for human and 36 and 48 h for the mouse lymphocytes) and a range of exposures (25–250 R). It was found that:

(a) For human lymphocytes, the dicentric yields with the 42 h fixation time were higher for two donors while these were nearly the same as those from a different donor for the lymphocytes of whom were fixed at 48 h;

(b) When the yields at the later fixation times were corrected appropriately by assuming that a cell containing a dicentric without an accompanyingacentric fragment was at its second mitosis after treatment, the yields at the 42 h fixation time was still higher; this suggested to the authors that the increased yield at the earlier fixation time was not entirely due to the fact that only the first division cells were being analysed;

(c) In mouse lymphocytes, the dicentric yields were higher at the earlier (36 h fixation time) than at the later one (48 h); when corrections similar to those carried out for human lymphocytes were made for second division cells, again the frequencies were still higher at the 36 h than at the 48 h fixation time;

(d) Considering all data together, it appeared that the dicentric yields in human lymphocytes were from 1.5 to 2 times higher than in those of the mouse, thus substantiating their earlier contention.

467. A further repeat of this work by Van Buul and Natrarajan [B74] with blood from 8 different human donors, x-ray doses of 1 or 2 Gy, fixation times of 42 and 48 hours in two experiments and several fixation times (40–60 hours) and PFG-staining in another, demonstrated that the "mixing-up" of first and second cell cycle at later sampling times cannot explain the observed variation in the frequencies of chromosome aberrations, but that donor donors variations is a predominant factor influencing aberration yields. In fact, the condition of the donor seemed to be most important since repeats on the same donor showed marked variability in sensitivity. Whether the inter-donor variations are due to different proportions of sub-populations of lymphocytes with different radio-sensitivities could not be answered. Confirmatory results showing that the frequencies of radiation-induced dicentrics were nearly the same at different fixation times when corrected for cells in their second and third mitoses were also obtained by Léonard and Debat [L26] and Scott and Lyons [S116]. In using blood from four different donors, the latter authors noted that in one donor, the frequencies of radiation-induced dicentrics were significantly different between two experiments.

468. In the study of de Boer et al. [B40] dealing with radiosensitivity of mouse and human lymphocytes to the induction of chromosome aberrations considered earlier, some results on the induction of dicentrics in lymphocytes of normal mice, mice heterozygous for a translocation between chromosomes 1 and 13, trisomic mice carrying the same translocated chromosome and either normal or abnormal in appearance were also presented. One of the findings was that these abnormal ("teeth trisomic") mice manifested a higher level of radiosensitivity than normal mice. However, in their recent work, Van Buul and de Boer [B69] could not confirm the above observation. They point out that since the trisomic mice occur in a variety of phenotypes and the splitting up into "normal-looking" and "teeth trisomics" is based on somatic properties only, it is likely that the genetic factor(s) that might have caused the high frequencies in the earlier study were missing in the "teeth trisomic" mice used in the later experiments.

469. Another mammalian species from which conflicting results had been obtained in the past is the rabbit. Scott and Bigger [S63] and Muramatsu and Matsuoka [M44] observed that the yields of x-ray induced dicentrics in the rabbit lymphocytes were less than one-half of those in human lymphocytes; Bajerska and Liniecki [B41] found however, that the sensitivities were the same (both species have the same effective chromosome arm number). With a fixation time of 48 h Léonard et al. [L29] found that the sensitivity of rabbit lymphocytes is in fact less than one-half of that of human lymphocytes. This finding was confirmed by Liniecki et al. [L28] who had earlier reported similar sensitivities of rabbit and human lymphocytes (1 to 4 Gy of x-rays; 40 h fixation time for the rabbit and 44–48 h fixation time for human lymphocytes). In the same study Liniecki et al. also assessed the sensitivity of pig
lymphocytes to the induction of dicentrics (1–4 Gy of x rays) and found (despite the fact that the chromosomes of the pig have 64 effective arms) that this was similar to that of rabbits (31 h fixation time for pig lymphocytes).

470. The recent and more decisive experiments of Fabry and Léonard [F13] using BUDR-Giemsa techniques (which permit unequivocal distinction between cells in M1, M2 and M3) demonstrated that there exists no significant differences in the frequencies of dicentrics in human and rabbit lymphocytes (200 R of x rays) provided that only cells in the first division were included in the calculations. Their results further show that at 48 h (the fixation time used in most of the studies on rabbit lymphocytes published in the literature and referred to in the preceding paragraph) around 50% of the cells are in the second and about 30% of the cells in the third division.

471. The finding of Muramatsu and Matsuoka [M44] discussed in the 1977 report, namely, that the lymphocytes of cat (arm number: 71) manifested only about one-fifth of the radiosensitivity of human lymphocytes to the radiation-induction of dicentrics, has now been confirmed by Stephan et al. [S66]. These authors found that the ratio of the yields of dicentrics in human as compared to cat lymphocytes is 1.0 : 0.27 (100–400 R).

472. In primates, Léonard et al. [L29] studied the chromosomal radiosensitivity of lymphocytes from the chimpanzee (Pan troglodytes), a hominoid ape phylogenetically and chromosomally closely related to man (48 chromosomes and 81 chromosome arms). No significant differences were observed in the radiation-induced frequencies of dicentrics and fragments (100–400 R; 48 h fixation time for both). The mean areas covered by the lymphocyte nuclei are, however, very different for the two species: 48.4 μm² for man and only 23.3 μm² for the chimpanzee. In the gorilla (Gorilla gorilla) however (chromosome arm number: 81) the sensitivity to the induction of dicentrics appears higher than in humans [D24].

473. Takahashi et al. [T15] compared the yields of dicentrics after acute and low dose rate gamma irradiation of the lymphocytes of man and the crab-eating monkey (Macaca fascicularis; 83 chromosome arms). Doses of 1–4 Gy were administered to heparinized whole blood samples at dose rates of either 0.5 Gy min⁻¹ or 0.17 Gy h⁻¹ (0.0029 Gy min⁻¹). With acute irradiation, the yields of dicentrics were similar in both species as had been found in an earlier study [H38]. At the low dose rate, the yields were lower in both species, as would be expected from the kinetics of induction of dicentrics (Y = aD + bD²). The surprising result was that, except at 1 Gy, the yields were significantly lower with monkey lymphocytes. A comparison of the values of the a and b coefficients after acute and low dose rate irradiation showed that:

(a) The magnitude of the linear components was not significantly different either between the species or between the radiation regimes;

(b) There was a drastic decrease in the magnitude of the quadratic component in the monkey (by a factor of about 30) whereas this reduction was much less with human lymphocytes (by a factor of 4).

474. These results permitted the authors to conclude that the chromosomal damage leading to dicentrics can be repaired in unstimulated (G₀) lymphocytes and that such repair capacity is different in the two species.

474. Takahashi et al. [T15] also looked into the question of whether there was any preferential elimination of cells with dicentrics during the course of low dose rate irradiation. This was carried out by incubating the cells for varying periods in the medium (2, 4, 6 and 24 h) after acute or chronic irradiation with 4 Gy prior to PHA stimulation. It was found that:

(a) With lymphocytes of both species, there was a decrease in mitotic indices and dicentric yields with post-irradiation incubation, although the time when these reached their lowest values was different (2 and 4 h after irradiation for mitotic index and dicentric yields, respectively); with the 24 h interval, the reduction was by one-quarter for dicentric yields for both the species while that for mitotic indices was by about 50% and 60% for man and monkey, respectively;

(b) At comparable time intervals between irradiation and PHA stimulation, the yields of dicentrics were similar in both the species;

(c) There was no evidence for preferential elimination of cells with multiple dicentrics in either species.

These results, particularly those showing species-independence for the elimination of aberrations with time (post-irradiation incubation prior to PHA stimulation), strengthen the view that the different yields of dicentrics obtained after low dose rate irradiation in the two species may be a reflection of primarily a species-specific G₀ repair mechanism.

475. Takahashi et al. [T16] have now extended their studies to the induction of chromosome aberrations at low gamma-ray doses (0.05 to 0.5 Gy; 6 levels). The methods used (48 h cultures; BUDR technique) were the same as in the earlier work. The results showed that as in the earlier work, there were no differences between the monkey and human lymphocytes with respect to the induction of dicentrics. The human data gave a satisfactory fit to a linear model (i.e., a linear increase in aberration frequency with dose), whereas this was not the case with those for the monkey. There was some suggestive evidence for the existence of a plateau in aberration yields between 0.1 and 0.3 Gy for the monkey and between 0.2 and 0.3 Gy for human lymphocytes, but more data would be needed to verify this suggestion, particularly for human lymphocytes.

3. Molecular mechanisms involved in the production of chromosome aberrations

476. In the 1977 report, some of the available information on the relationship between radiation-induced lesions in the DNA, their repair and their relationship to mutations and chromosomal aberrations was reviewed. Some new data have been collected since then which shed further light on the mechanisms involved in the formation of chromosome aberrations in eukaryotic systems, and these will be reviewed in this section.

477. Kihlman et al. [K26] used the 5-bromodeoxyuridine labelling technique to explore the mechanisms involved in the formation of chromosomal aberrations. The rationale for using this technique is the following: the incorporation of BUDR into the DNA of a chromatid alters its staining properties and its sensi-
itivity to chromosome damage by UV and x rays. A chromatid that has incorporated BUdR into both strands of its DNA stains more weakly with the fluorescence plus Giemsa (FPG) technique than a chromatid that has incorporated the BUdR into only one strand of its DNA, and such a single-substituted chromatid in turn does not stain as strongly as a completely unsubstituted chromatid. The same dose of x rays produces about three times more breaks in unifilarly substituted chromatid than in an unsubstituted one. The extreme case is represented by long-wave UV (i.e., wavelengths of between 310 and 350 nm) which produces aberrations exclusively in chromatids having BUdR-substituted DNA.

478. Although the thesis that two lesions are required to produce one exchange aberration is not a new one in radiobiology (it was arrived at long ago on the basis of dose-effect relationship for radiation-induced exchanges [L38, M67, S78]) direct evidence has been lacking. The experiments of Kihlman et al. [K26] were designed to obtain such evidence.

479. Root-tip cells of Vicia faba were exposed in the G2 phase to long-wave (320–380 nm) UV radiation or to x rays (40 R); before the irradiation exposures, BUdR had been substituted for thymine in various numbers of DNA strands in these chromosomes. The experiments involved cells with chromosomes of the following constitution: TT-CT (both chromatids of the chromosome containing unsubstituted DNA), TT-TT (one chromatid with unsubstituted DNA and one with unifilarly substituted DNA). The sister chromatids in chromosomes of the TT-TB and TB-BB constitution could be distinguished by differential staining with the FPG technique.

480. The results show that:
(a) Long-wave UV induced no aberrations in chromosomes with unsubstituted DNA;
(b) In chromosomes with BUdR-substituted DNA, long wave UV, like x rays, induced subchromatid and chromatid aberrations; because these aberrations were found in the first mitosis after exposure of G2 cells, the effect of long wave UV was of the S-independent, ionizing-radiation-type;
(c) The sensitivity to the production of aberrations by x rays increased with increasing number of BUdR-substituted DNA strands, i.e., TT-CT < TT-TT < TT-TB < TT-TB < TT-BB;
(d) The increased sensitivity of TB-TB chromosomes over the TT-TB chromosomes was particularly striking.

481. The question of whether a damaged chromatid may interact with an undamaged one to form an exchange (as has been suggested in the model of Resnick [R54]) was studied by making use of the finding that in cells with chromosomes of the TT-TB constitution, long wave UV produced no breaks in TT chromatids, whereas x rays produced about three times as many breaks in the TB chromatids as in TT chromatids. In the latter case, if the assumption that two lesions are required to form one exchange is true, then, there should be nine times more exchanges between TB chromatids than between TT chromatids. If, on the other hand, one lesion can give rise to an exchange, there should be three times as many exchanges between TB chromatids as between TT chromatids. The results were in line with the first assumption. These results provide direct evidence that two lesions are required to produce one exchange.

482. Natarajan and Obe [N15] tested the hypothesis whether or not double-strand DNA breaks are responsible for radiation-induced chromosome aberrations. They made use of the finding of Tanaka et al. [T10] who demonstrated that the introduction of T4 endonuclease in Xeroderma Pigmentosum (XP) cells in vitro in the presence of inactivated Sendai virus led to the ability of these cells to perform unscheduled DNA synthesis. This indicated that, as XP cells are deficient in the first (incision) step of excision repair, this first step can be mediated by the endonuclease and when this is done, the other steps in the sequence can be carried out by the cells' own enzymatic machinery. According to the accepted model of excision repair, following treatment with a mutagen causing direct breaks (x rays) or where the repair processes generate single-strand breaks (UV), transient short stretches of single-strand regions should be present in the chromosomal DNA of the treated cells. If single-strand-specific endonuclease, such as Neurospora endonuclease, is introduced in these cells, one would expect to induce double-strand breaks in the single-stranded regions of the DNA. If double-strand breaks are responsible for radiation-induced chromosome aberrations, under these conditions, there would be an increase in the frequency of aberrations for a given dose in the ensuing mitosis.

483. CHO cells were irradiated with x rays or short-wave UV or treated with chemicals (MMS, bleomycin, mitomycin-C) in G2 stage in the presence of the inactivated Sendai virus and Neurospora endonuclease and the aberrations were scored in the ensuing mitosis. In the x-ray series, the above treatment led to an enhancement of the frequencies (by a factor of about 2 at the lowest exposure of 50 R and much higher at 100 and 200 R) of breaks, exchanges and gaps. These results have been interpreted by the authors as due to the conversion of some of the x-ray induced single-strand DNA breaks into double-strand breaks by the enzyme. Similar results were obtained after treatment with MMS and bleomycin but not after UV or mitomycin-C; with the latter two, however, there was a clear increase in the frequency of gaps.

484. The possible reasons for the difference in response between x rays, MMS and bleomycin on the one hand and UV and mitomycin C on the other are the following: ionizing radiations produce chromatid aberrations in G2 and the effect is S-independent. It does not require DNA and chromosome replication to be expressed as chromosomal aberrations. Bleomycin is known to act like x rays, i.e., to be S-independent. Although the aberrations induced by MMS are S-independent, MMS is known to behave more like x rays with regard to excision repair, namely, "small-patch" repair [R55], MMC, on the other hand, is a cross-linking bifunctional alkylating agent and the aberrations are of the delayed type and S-dependent. Short-wave UV induces dimers which are implicated as being responsible for UV-induced chromosome aberrations; these are of the S-dependent type. It is known that in addition to dimers, UV induces strand breaks. During excision repair of UV-induced lesions, long patches of single strand regions are expected to occur. It would appear that single-strand stretches following the "long patch" repair even if initiated in G2 are not converted into double-strand breaks by the endonuclease and it may be that this type of repair relevant to initiation of chromosome aberrations is confined to the S phase only.

485. In a subsequent study, Natarajan et al. [N16] extended their observations to CHO cells irradiated in
the G₁ stage of the cell cycle, in addition to examining the duration of availability of single-strand gaps for action by Neurospora endonuclease in irradiated G₂ cells. The results show that after G₂ irradiation, (50–200 R) all classes of chromatid aberrations increased, as noted in the earlier study. In G₁ cells (75–300 R) an increase in chromosome-type of aberrations was found but there was also a marked induction of chromatid aberrations. The increase in chromosome-type aberrations in irradiated G₁ cells treated with Neurospora endonuclease has been interpreted as due to the conversion of DNA single-strand breaks or gaps to double-strand breaks by the enzyme; the induction of chromatid aberrations in G₁ was assumed to be due to conversion of some of the damaged bases into strand-breaks by the enzyme. The authors have provided evidence for the conversion (by the enzyme) of single-strand breaks induced by x rays into double-strand breaks using neutral sucrose gradient centrifugation.

486. The data from experiments designed to determine how long the single-strand breaks or gaps produced in G₂ by x rays remain available for action by the enzyme show that immediate post-irradiation treatment with the enzyme leads to a marked increase in the frequencies of chromatid gaps, breaks and exchanges. Later post-treatments, i.e., after 60 or 90 min, increase the frequencies only of breaks, but not of exchanges. It would thus seem that single-strand regions are available even 60 min after irradiation, but the induced double-strand breaks are not able to interact effectively to produce chromatid-type aberrations. This may be due to changes in spatial arrangements of chromosomes in later G₂ or prophase cells prior to mitosis, which restrict interaction of breaks.

487. Kato [K27] demonstrated that the frequencies of UV-induced sister chromatid exchanges (SCE) in rat kangaroo cells were reduced on photoreactivation with visible light, suggesting that pyrimidine dimers are involved in the production of SCEs. Reynolds, Natarajan and Lehman [R56] irradiated CHO cells with far UV and near UV (broad-spectrum) and determined the frequencies of SCEs over a range of doses; in parallel experiments, enzymatic assays were performed to assess the numbers of pyrimidine dimers making use of dimer-specific endonuclease from Micrococcus luteus. It was found that both types of UV irradiation induced SCEs, which showed a linear increase with exposure time (dose); in addition, the enzyme specific sites (ESS) showed a similar increase and the ratios of SCEs to ESSs were nearly the same over the dose range studied. These data thus suggest that dimers are related to SCE induction. However, a substantial number of dimers need to be produced in the DNA of CHO cells before one SCE can occur and their calculations show that about 20 000 pyrimidine dimers need to be induced for each additional SCE.

488. Recently, in experiments with chick embryonic fibroblasts (which possess photoreactivating enzymes), Wolff [W22] found that photoreactivation (PR) following UV irradiation leads to the disappearance of dimers but not of SCE's. The interpretation was that some other minor photoprocess and not the dimers may be responsible for the induction of SCE's. Similar results were obtained by Natarajan et al. [N17] after 5 and 10 J/m² UV irradiation followed by PR; there was a reduction in the number of dimers (i.e., ESS) but no reduction in the frequencies of SCEs. The frequencies of SCEs seemed to saturate around 20 per cell.

489. An examination of the chromosome preparations showed that at these UV doses, there was a considerable mitotic delay and a high frequency of chromosome aberrations in cells which were still in their first mitosis. This would indicate that only a small proportion of cells came through to a second cell cycle. In subsequent experiments therefore, Natarajan et al. used lower UV fluences (1 and 2 J/m²). The results showed that:

(a) There was a considerable amount of dimer removal (by about 95%) after PR;
(b) There was a reduction in the frequencies of SCEs by about 50%.

490. In order to investigate the effect of fixation time on the frequency of SCEs, an experiment with 2 J/m² was conducted and the cells were fixed at 5 h intervals between 24 and 35 h. The data clearly demonstrated removal of dimers with PR (to the extent of about 85%) and that, at all fixation times, there was a significant reduction in the frequencies of SCEs which varied between 25 and 40%. These results, therefore, show that there is a relationship between SCEs and pyrimidine dimers. The discrepancy between these data and those of Wolff [W22] is probably due to the possibility that high UV fluences were used in his study.

491. Using Potorous cell line PtK2, Ishizaki et al. [I18] found that the frequency of UV-induced SCEs could be reduced by post-irradiation exposure to visible light. In their experiments, UV fluences of 5 and 10 J/m² were used. Moreover, the effect of PR light was temperature-dependent; the authors therefore concluded that the reduction in the frequency of SCEs is probably mediated through an enzymatic reaction.

492. In more recent work, van Zeeland et al. [Z11] conducted experiments with fibroblasts from Xenopus laevis which possess photoreactivating enzyme to study the influence of photoreactivation on UV-induced pyrimidine dimers, sister-chromatid exchanges, cell-killing and point mutations to ouabain resistance. The results clearly showed that the frequencies of all the biological endpoints studied were reduced on photoreactivation and this was paralleled by a decrease in the frequencies of pyrimidine dimers (determined as endonuclease sensitive sites). However, an absolute quantitative relationship could not be established between the photoreactivation-mediated reduction in the amount of pyrimidine dimers and the decrease in the frequencies of SCEs, chromosomal aberrations, point mutations and the extent of cell killing and this could be due to a number of causes. Nonetheless, these studies show a direct relationship between biological effects and UV-induced pyrimidine dimers in this system.

4. A test of the hypothesis of whether there is proportionality between spontaneous and induced rates of mutations

493. One of the main assumptions involved in the use of the doubling-dose method for risk evaluations in man is that of proportionality between the average spontaneous and induction rates of mutations. An example will serve to illustrate this point. Suppose that in species A, the average spontaneous rate is 10⁻⁵ per gene and the average rate of induction is 10⁻⁷ per unit dose per gene. The doubling dose then is 100 dose units and the mutational risk per unit dose of radiation exposure is 1/100 of the spontaneous incidence. Suppose that in a related species B, the average
spontaneous rate is lower, say $10^{-6}$ per gene. It will be valid to apply the doubling dose of 100 dose units to species B only when it is assumed that the average rate of induction in species B is also lower, say, $10^{-8}$ per unit dose per gene such that $10^{-2}/10^{-8} = 10^{-6}/10^{-8} = 100$. If for instance in B, the rate of induction is $10^{-7}$ (i.e., the same as in A), the doubling dose will be 10 dose units and the mutational risk will be an order of magnitude higher, namely $1/10$ of spontaneous incidence (instead of $1/100$ of the spontaneous incidence) in spite of the fact that the amount of damage induced will be the same in both cases.

494. One way to test the validity of the assumption of proportionality between spontaneous and induction rates is to examine the average induction rates of mutations in related species or strains of organisms that differ from one another in their spontaneous rates and see whether such a proportionality is in fact obtained. Another method is to examine within a species whether or not such a proportionality between spontaneous and induction rates is obtained on a locus-by-locus basis for a number of gene loci. Shukla, Sankaranarayanan and Sobels [S79] adopted this latter approach to study this problem in Drosophila melanogaster. These investigators collected data on the induction of specific-locus mutations at 14 X-chromosome loci following spermatogonial irradiation (x-rays; 3000 R). The mutational spectrum obtained in the radiation work was compared with the spectrum that Schaefer [S80] has observed in his work on spontaneous mutations using the same set of loci and stocks. The conclusion that emerged from their work was that the data were not out of line with the assumption of positive correlation between spontaneous and induction rates of mutations for the loci under study but that more extensive data, both on spontaneous and induction rates would be required before the evidence could be considered conclusive. Furthermore, a proportionality across the spectrum of mutational rates for spontaneous and radiation-induced mutations in spermatogonia may not hold for spermatogonia, since it has been shown in the mouse that the spectra for induced mutations in these two germ cell stages are not the same.

495. In another study, Racine, Langley and Voelker [R37] accumulated enzyme mutants by exposing a balanced lethal strain of Drosophila melanogaster (heterozygous at seven allozyme loci) to chronic gamma irradiation (0.17 Gy h$^{-1}$) for 14 generations (15% daily total accumulated dose: 335.52 Gy). Following the 14 generations of exposure the flies were screened by gel electrophoresis for newly arisen null mutants and/or mobility variants. Among other things, the rank order of mutability observed in this work was compared with the one recorded by Mukai and Cockerham [M3] and their own unpublished results on spontaneous mutations at the same set of loci. This analysis revealed the following rank order for radiation-induced mutations (the numbers of mutants are shown in parenthesis): αGpdh (6); Hex-C (4); αAmy (0). For spontaneous mutations, the order was the following: βGpdh (7); Hex-C (5); cMdh (2); Got-2 (1); Adh (0); Dip-A (0) and αAmy (0). For spontaneous mutations, the order was the following: αGpdh (6); Hex-C (4); cMdh (2); Got-2 (1); Dip-A (0) and Adh (0). It can be seen that there is a good correspondence between the two rank orders suggesting that these data are consistent with the assumption of proportionality between spontaneous and induced mutations.

496. Glickman et al. [G33] conducted a study on gamma-ray induction of mutations in the lacI gene of Escherichia coli, a locus which codes for the lac repressor. Exposures from 5 kR to 40 kR were used and the lacI mutants recovered (among which were nonsense mutations ranging from 1.4 to 10.8% in the different experiments) were analysed for changes at more than 70 nonsense sites (amber and ochre) within the lacI gene. Contemporary controls were run and the conclusions drawn pertained to 98 and 135 independent nonsense mutations isolated in the controls and 30 kR radiation experiments, respectively.

497. Among the spontaneous mutants, both transitions and transversions were frequent and there were three "hot spots" in the amber spectrum at sites 6, 15 and 34. These sites had previously been shown by Miller et al. [M48] to correspond to the location of 5-methylcytosine residues in the DNA; this naturally-occurring modified DNA base gives 5-methyluracil (thymine) by spontaneous deamination and therefore occasionally results in errors in the DNA that go uncorrected by uracil-N-glycosidase [C39]. Gamma rays also induced both transitions and transversions but the prominent amber "hot spots" seen with the spontaneous mutants were absent, suggesting that gamma rays presumably do not cause extensive deamination. Transitions constituted 55.6% of the gamma-ray induced amber mutants compared with 71.4% transitions among the spontaneous amber mutants; the difference is statistically significant at the 5% level. When the amber data were analysed again with the amber "hot spots" excluded, the distribution of transition and transversion events in the spontaneous and induced mutants was similar. The authors have drawn the conclusion that the spectra of induced and spontaneous mutations are similar with the exception of the spontaneous "hot spots".

5. Chromosomal evolution in primates and its possible relevance for inter-specific comparisons and for assessing the chromosomal basis of human pathology

498. Dutrillaux [D4] has published an exhaustive review of the chromosomal evolution in primates and has proposed a tentative phylogeny from Microcebus murinus (a Prosimian which seems to have retained a rather primitive karyotype) to man. The chromosomal studies were carried out after whole blood culture or fibroblast culture after skin biopsy. Detailed comparisons of the karyotype of man with those of about 60 species (of the approximately 200 species of the order of primates) were made, on a band-by-band basis for every chromosome using all the available banding techniques. Almost all the data used for these comparisons have been collected by the author and his colleagues. The major conclusions of use to the Committee that emerged from this work are summarized in the following paragraphs.

499. To facilitate discussions, the reconstructed evolutionary tree showing the sequence of chromosomal changes that have occurred is diagrammed in Figure II. An examination of the above figure shows that the pattern of chromosomal evolution is not the same in the different lines. The types of rearrangements that have occurred vary from one group (suborder, family, genus)
to another. For instance, Robertsonian translocations are preponderant among the Lemuridae (44 of the 57 changes are of this type), but are non-existent among the Pongidae. Chromosome fissions are very frequent among the Cercopithecinae (26/51, including new information), but are not found elsewhere, and pericentric inversions are preponderant in the evolution of Pongidae and the human species (17/28).

500. There is a very close similarity of chromosome banding patterns between the simians studied and man. All the quantitative and some of the qualitative variations detected involve the heterochromatin. Approximately 70% of the bands are common to the simians and to the lemurs (prosimians). In the remaining 30%, technical difficulties prevented a meaningful comparison but this does not exclude the possibility that a complete analogy may exist. It thus appears that the chromosomal evolution of the simians (and probably of all the primates) has occurred without detectable duplication or deficiency of the euchromatin.

501. The reasons why particular kinds and/or sequence of changes have occurred in one but not in another line can only be conjectured but the answers presumably lie in the breeding structure and size of the evolving populations, the selective advantages or disadvantages of given rearrangements, the genic contents of the chromosomes concerned, the functional alterations that may accompany structural alterations (position effect), etc. The available data suggest that of all these considerations, evolution by structural-change-mediated position effects appears least likely.

502. As mentioned earlier, the kinds and relative frequencies of the different rearrangements are different in the different lines of evolution. For instance, a closer look at Figure II, segment B→HSA will reveal that from the last common ancestor of all simians to man, 50% of the changes are pericentric inversions, 13% complex intrachromosomal rearrangements and 16% complex, imprecisely-defined interchromosomal ones. In the direct ancestors of man (segment D→HSA; Figure II) again, 8 of the 15
changes are pericentric inversions, the remainder being made up of others (3 other intrachromosomal, 3 interchromosomal and 1 paracentric inversion) with no Robertsonian translocations.

503. In contemporary human populations, as was discussed in chapter I, the two kinds of rearrangements that are predominant are the Robertsonian and reciprocal translocations; the pericentric inversions constitute a minor class. It would thus appear that the categories of rearrangements most frequently observed in humans are not those that occurred in the evolution of our ancestors.

504. Some of the types of chromosomal changes that have been noted during the evolution of primates are also known in chromosomally abnormal human individuals. Apart from the Robertsonian and reciprocal translocations mentioned earlier, pericentric inversions appear to be common to both human evolution and human pathology, although the relative frequencies are different. It would therefore appear instructive to examine whether these inversions that are rarely observed in man are not those that are “frequently” observed in evolution. In the work reported [D4], Dutrilliaux could identify 44 pericentric inversions among which 21 occurred either on chromosomes that were already identical to those of man or on those which were at the last stage before giving rise to human chromosomes. Two-thirds of the latter could be considered as possible “convergent mutations” (in this context, referring to the occurrence of similar structural changes in species belonging to different lines of descent) and one-third, “reverse mutations” (the occurrence of structural change leading to ancestral chromosome(s)). From the data available for patients (over 10,000 of them) at the Institut de Progénèse, Dutrilliaux could extract 26 independent pericentric inversions; in addition, there were three independent cases of paracentric inversions.

505. A comparison of these 29 inversions with those detected during evolution permitted the conclusion that 8 of the former could be considered as possible “reverse mutations” and 1 a possible “convergent mutation”. Of these 8, 7 were pericentric inversions and 1 a paracentric inversion; out of the first 8, 1 was observed 3 times and another 2 times. Considering the fact that the number of breakpoints and consequently the number of different inversions is theoretically very large, it would appear that the correlation between changes that have occurred in human pathology and in human evolution is more than mere coincidence.

506. The implications of these findings for interspecific comparisons of chromosomal sensitivity to radiation (or other mutagens) to the induction of different kinds of aberrations can only be speculated on at present. Two questions, yet unresolved, are: “can the evolutionary history provide any clue with respect to the inducibility of a given kind of chromosomal aberration in different species and in man and the pathological consequences that may derive from such induced aberrations in man?” and “can quantitative and qualitative comparisons be based on the induction of one kind of aberration in different species provide a proper perspective and a basis for predicting the relative radiosensitivity of human chromosomes to damage that is relevant in the context of induced pathological states?”

6. Summary and conclusions

507. Experiments were conducted in which mice and rabbits were kept in huts in sites of high concentrations of uranium in the soil. The radiation exposures were from external gamma-irradiation and from inhaled radon. Periodical blood samples were taken from the rabbits to assess the amount of chromosome damage; the mice were used in tests on fertility and for cytogenetic studies on induced reciprocal translocations (spermatoocyte analysis).

508. In the rabbits, the frequency of dicentrics in the lymphocytes rose from 0.00 per cent (at the beginning of the experiment) to 1.9% after 4 months exposure (gamma-ray doses in the range from 0.13 to 0.27 Gy for individual rabbits), to 2.6% after eight months (gamma-ray doses of between 0.15-0.53 Gy for individual rabbits) and to 3.9% after one year. After a 16-month period, there was no further increase in the frequency of aberrations and after a 20-month period, the frequency actually dropped to zero. The radon exposure estimates were 5-10 Gy per year to the bronchial region and about 1 x 10-3 Gy per year to the gonads. From model studies with radon exposures alone to rabbits, it was concluded that the chromosome aberrations observed were all due to gamma irradiation.

509. Male mice kept in the radiation area for three months showed slight increases in litter size, the mean number of offspring sired and in the mean number of offspring weaned. Female mice (also kept in the radiation area) showed slight reductions in all the above three indicators of radiation effects. Neither the irradiated male mice nor their sons showed any evidence for the presence of translocations or other aberrations in their spermatocytes. The estimate of the external gamma-irradiation for the 3-month period was 0.15 Gy.

510. Additional data on the radiation-induction of aberrations in lymphocytes (dicentrics) that have become available since the publication of the 1977 report confirm the conclusion reached by the Committee in its 1977 report, namely, that there is no simple relationship between the effective chromosome arm number of the species and its relative radiosensitivity to the induction of these structural aberrations. The mammalian species studied in this respect included the rabbit, the cat, the chimpanzee, the gorilla and the crab-eating monkey. Furthermore when it is ensured that only cells in their first mitosis after irradiation are scored for dicentrics, there are no differences in sensitivity between mouse and human lymphocytes, and between rabbit and human lymphocytes.

511. Whereas after acute gamma irradiation the frequencies of radiation-induced dicentrics in the lymphocytes of man and the crab-eating monkey are similar (at similar doses) after chronic irradiation, the yield in monkey lymphocytes was lower. The difference after chronic irradiation has been explained on the assumption that chromosomal damage leading to dicentrics can be repaired in unstimulated (G0) lymphocytes and that such a repair capacity may be different in the two species.

512. Direct evidence for the thesis that an exchange aberration is the result of interaction of two lesions has been obtained using root-tips of Vicia faba in which prior to irradiation with long-wave UV or x rays, BUDR
was substituted for thymine in various numbers of DNA strands in the chromosomes with a consequent change in radiosensitivity, this increasing with increasing numbers of substituted strands.

513. Data demonstrating that double-strand breaks in the DNA are responsible for radiation-induced (x-ray) chromosomal aberrations has been obtained in CHO cells in vitro. The experiments involved the introduction of Neurospora endonuclease into cells made permeable prior to the x-ray exposure. The results showed a marked increase in the frequency of aberrations under these conditions relative to controls which were not treated with the enzyme. Similar results were obtained with bleomycin and MMS, but not with UV or mitomycin-C. Possible reasons for these differences are discussed. In the x-ray work, there is evidence showing that the single-strand breaks produced by irradiation are available for action by the enzyme even 60 minutes after irradiation, but the induced double-strand breaks are not able to interact effectively to produce chromatid-type aberrations as the interval between irradiation and the enzyme treatment increases.

514. The frequencies of UV-induced sister-chromatid-exchanges in rat kangaroo cells and in a porcous cell line are reduced on photoreactivation with visible light, suggesting that UV-induced pyrimidine dimers are involved in the process of DNA repair. Using a similar technique, the involvement of dimers in UV-induced SCEs has also been demonstrated in CHO cells.

515. In chick embryonic fibroblasts (which have the PR enzyme), PR following UV-irradiation led to the disappearance of the dimers but not of SCEs in some studies. The reason for this has now been clarified: higher UV fluences which were used in these studies led to a considerable mitotic delay and, consequently, only a small proportion of cells came through to a second cell cycle. With lower UV fluences, clear evidence has been obtained for removal of dimers by PR treatment after UV as well as for a reduction in the frequencies of SCEs.

516. The hypothesis of whether there is proportionality between spontaneous and radiation-induced mutation rates (an assumption which is central to the use of the doubling-dose method for risk evaluations) has been tested in Dro sophila and in E. coli. In Dro sophila, the data on spontaneous and induced mutation rate estimates for a number of individual loci are consistent with the hypothesis. In E. coli where the comparison was between spontaneous and induced mutation rates at more than 70 sites in the lacI gene, if the three amber "hot-spots" (predominant in the spontaneous mutation spectrum) were excluded, there was correspondence between spontaneous and induced mutation rates for the different sites.

517. Extensive data on chromosomal evolution in primates have been published in which detailed comparisons of the karyotype of man with those of about 60 primate species have been made (on a band-by-band basis for every chromosome using all the available banding techniques). It has been found that the pattern of chromosomal evolution is not the same in the different branches of the evolutionary tree (i.e., the types of rearrangements that have occurred vary from one group to another). From the last common ancestor of all simians to man 50% of the changes are pericentric inversions. However, in contemporary human populations, the pericentric inversions constitute a minor class. The possible implications of the finding of pericentric inversions in chromosomally abnormal individuals are briefly outlined within the framework of the evolutionary history of our species.

III. EFFECTS OF X-IRRADIATION ON SURVIVAL KINETICS OF SPERMATOGONIAL CELLS IN MICE AND RATS

518. Oakberg [O10] conducted a study to investigate the effects of x-irradiation on the survival of stem cell spermatagonia in the mouse and to obtain dose-response curves for both single and fractionated exposures in order to determine if the cell populations surviving these treatments are qualitatively different. This study is an extension of the author's earlier work [O11] discussed in the 1972 and 1977 reports of the Committee.

519. One group of adult C3H x 101 hybrid male mice received three injections of 0.46 MBq or 3H-thymidine at 9 h intervals and x-irradiation (100, 300, 500, 600, 1000 R or the first half fraction of a 1000 R exposure) 24 h after the last injection. The mice were killed 120 h and 207 h (in one experiment, 414 h) after irradiation. The rationale for choosing 120 and 207 h is that it is known from previous work [O12] that some surviving stem cells will divide between these two intervals, although intervals of five days or more are required for the process of degeneration to run its course. A second group of mice received a single injection of 0.46 MBq of 3H-thymidine one hour before x-irradiation at levels similar to those mentioned above. Appropriate controls were maintained. Tissues preparations were made for autoradiography and for scoring of survival of the stem cells; cell survival was measured by counting stem cells in 99 tubule cross sections per slide (198 per mouse) distributed among the stages of the cycle of the seminiferous epithelium: labelling was scored by counting 100 A spermatagonia per slide (200 per mouse).

520. The stem-cell survival data 120 and 207 h after x-irradiation are given in Table 35. The mean number of A cells in the unirradiated mouse is reduced by the injection of 3H-thymidine and likewise, irradiated mice given 3H-thymidine show lower cell counts than mice that received radiation alone. Compared to the 12 h data, the number of cells scored in the unlabelled group (experiment 1) after 207 h is higher at 300, 500 and 600 R, lower at 1000 R and the same for the 500 + 500 R exposure; in the mice receiving 3H-thymidine survival after 207 h is higher than after 120 h at 300, 500 and 600 R, and lower for both 1000 R and 500 + 500 R exposures. At both intervals, with and without 3H-thymidine, the data fit a curvilinear relationship with no evidence of discontinuity over the 100–1000 R range, though it should be noted that there are no points between 600 and 1000 R.

521. The data from experiments on the labelling of A spermatagonia are summarized in Table 36. The percentage of labelled cells at 207 h is markedly influenced by the total exposure and by exposure fractionation; for instance, in the 3 x 0.46 MBq group, the percentage of labelled cells does not differ significantly between controls and the 100–600 R groups, is significantly lower in the 1000 R group and significantly high in the 500 + 500 R group. At 414 h, there is no significant difference between 100, 300, 500, 600 and 500 +
500 R groups (all are higher than controls) while the single 1000 R exposure is lower than in the controls just as at 207 h. When irradiation is given 1 h after $H^3$H-thymidine injection, very few labelled cells survive any of the exposures used with values ranging from 2.2% to 6.6% compared with 21% for controls.

522. From these results, Oakberg [O10] has concluded that the surviving stem cell population is qualitatively the same for that portion of the dose-response curve giving a linear increase in mutation frequency, but different for both 1000 R single and fractionated exposures. This parallelism between survival of labelled cells and mutation frequency in spermatogonial stem cells summarized in Table 37 suggests that a stage in the cell cycle A relationship after DNA synthesis is resistant to cell killing but sensitive to mutation induction. The mutation rate after a single 1000 R exposure is low because labelled mutation-sensitive cells have been selectively killed. The mutation frequency after the 500 R exposure is increased because of synchronization induced by the first fraction combined with the selective killing of unlabelled cells by the second one. Irradiation one hour after the labelling with $H^3$H-thymidine demonstrated that the S phase of the spermatogonial stem cell cycle is sensitive to radiation-induced cell killing.

523. There are some interesting differences between the dose-response relationship reported by different authors for effects of low-LET irradiation on some direct measures of spermatogonial survival. As already stated, Oakberg [O10] found a curvilinear (quadratic) response for survival of type A4 spermatogonia scored 207 h after x-irradiation, with no sign of discontinuity at 600 R. De Ruiter-Bootsma et al. [R76] reported an exponential relationship over the range for spermatogonial stem-cell survival measured 3 weeks after irradiation by the tubule repopulation method, again with no sign of discontinuity around 6 Gy.

524. Cattanach [C40], however, reported an exceptionally high 600 R point (giving a plateau between 600 R and 700 R) in the otherwise exponential dose-response curve for the median day of return to fertility (i.e., length of the sterile period which results from spermatogonial killing) after 500-1000 R x-irradiation. He interpreted this as due to the presence of two stem-cell populations with different radiosensitivities to radiation killing. Lu et al. [L48] assayed levels of the X-isozyme of lactate dehydrogenase, LDH-X (providing a measure of the number of spermatocytes plus spermatids) and numbers of sperm-heads in testicular homogenates 56 days after 0–14 Gy of acute gamma-irradiation. Both curves had very large shoulders with inflection points at 5 and 6 Gy. The authors concluded that there was a radiosensitive subpopulation of stem-cells which predominated after doses greater than 6 Gy.

525. Thus, results of the two histological assays are in agreement with each other, as are those of the two functional assays. As the former two assays measured the stem-cell survivors at an earlier post-treatment stage than the latter two, the possibility arises that some temporal factor (possibly connected with a feed-back mechanism) alters the form of the response between the initial phase of spermatogonial proliferation and the final phase of spermatogonia. Oakberg [O10] suggested that the 600–700 R plateau in return to fertility found by Cattanach might be connected with the dynamics of repopulation at higher doses when the number of surviving colonies is low. This might affect the return to sperm numbers adequate for fertility in a manner which is dose-dependent. However, this hypothesis may need modification in view of the more recent results of Lu et al. [L48] already discussed.

526. Huckins and Oakberg [H59, H60, H61] have recently carried out further analyses of the behaviour of stem-cell spermatogonia in normal and irradiated rat and mouse testes, mainly using whole mounts of seminiferous tubules. When their conclusions are compared with those of Cattanach and co-workers [C32, C63, C64, C65] derived from translocation studies and already discussed in section II.B., a consensus seems to emerge. The only spermatogonia which survive an acute exposure of 100 R or more (apart possibly from some A1 cells) are some of the radioresistant long-cycling stem cells.

527. According to Huckins and Oakberg [H61] these survivors are likely to be in a prolonged "protective" G1 phase, while Cattanach and Crocker [C63] have described it as G0 phase. Probably as the result of the depletion of other spermatogonial stages, these long-cycling surviving cells are triggered from the indeterminate "A-phase" into the determinate "B-phase" [SI18] so that mitotic proliferation proceeds. The time from entry into DNA synthesis 2 to 3 1/2 days later [H61]. Most stem-cells then go into a short cycle, as the results of de Ruiter-Bootsma and colleagues indicate [R76]. Some stem-cells start to differentiate into subsequent stages before the complete rebuilding of the undifferentiated population [H61].

528. At 24–48 h after irradiation the surviving A4 spermatogonia synchronously arrive at what is probably a transition phase sensitive to the induction of mutations and chromosome aberrations, but in which some cells could still survive a large radiation dose. The sub-additive mutational response at longer fractionation intervals (3–15 days) presumably reflects the sensitivity of the short cycling stem-cells. These are the only type found in the immature testis, which also has a reduced radiation response [C64, E22, S119]. The exact relationship between the long-cycling and the short-cycling stem-cell spermatogonia is still obscure. It is possible that the short cycle precedes differentiation, or that short-cycling cells periodically and randomly enter a longer cycle [H61].

529. Although the long- and short-cycling cells can be regarded as more resistant and more sensitive subpopulations, it seems difficult to equate them with the entities thus described by Cattanach et al. [C40] and Lu et al. [L48] since the shorter cycling cells seem to disappear at doses which are decidedly lower than the > 6 Gy postulated. However, it seems clear that heterogeneity in sensitivity to killing against the induction of mutations in spermatogonia must remain within the long-cycling A4 cells at stage G0 or G1 which would alone survive doses of 6 Gy or more, since otherwise it would be very difficult to explain the humped type of dose-response curve for the induction of point mutations or translocations. The exact nature of the mechanism which triggers active stem-cell proliferation after irradiation is still unknown, but it should be noted that neither Cattanach et al. [C64] nor Cunningham and Huckins [C66] have been able to demonstrate a chalone effect in the irradiated testis.

530. In the 1977 report, the work of Hsu and Fabrikanth [H45] on the cellular response and cell population kinetics during spermatogonial cell renewal
in the mouse testis exposed to continuous gamma irradiation at 1.8 $10^{-2}$ Gy d$^{-1}$ and at 0.45 Gy d$^{-1}$ was described. The results were that:

(a) At the lower dose rate for seven days, the A$\alpha$ spermatogonial cells showed no reduction in cell number; at the higher dose rate, the number of A$\alpha$ cells was reduced to 50% of that in controls during the first three days of continuous irradiation and from day 4 to 10, its figure was 80%. From day 11 onwards, control levels were reached;

(b) In the group irradiated at the higher dose rate for two weeks, the repopulation of the seminiferous epithelium commenced with increased cell proliferation in the A$\alpha$ cells. This was supported by the finding of an increase in the A$\alpha$ mitotic index. Labelling with $\gamma$-thymidine was used in this study for the analysis of cell population kinetics.

531. Erickson [E14] has recently reported the results of a similar study in rats with a broader spectrum of low dose rates but without using any radioactive label. Sprague-Dawley rats were irradiated continuously with $\gamma$Co gamma rays at dose rates of $1 \times 10^{-2}$, $3 \times 10^{-2}$ or $6 \times 10^{-2}$ Gy per 23 h day ($7 \times 10^{-6}$ Gy min$^{-1}$, $2.1 \times 10^{-5}$ Gy min$^{-1}$ and $4.2 \times 10^{-5}$ Gy min$^{-1}$) for periods ranging from one month to six months. At the termination of the radiation treatments, the different groups, the animals were killed and testes preparations were made. Spermatogonia were counted in whole mounts of seminiferous tubules that were isolated from the testes.

532. At $1 \times 10^{-2}$ Gy d$^{-1}$, there was no statistically significant effect on stem cell number and this was so irrespective of the duration of exposure (i.e., irrespective of the total dose delivered); at $3 \times 10^{-2}$ and $6 \times 10^{-2}$ Gy d$^{-1}$, there was an initial drop (after one and two months of irradiation, respectively) to about 80 or 60% of the control level, followed by a slower decrease reaching values of 60 and 40% of the controls.

533. One month after irradiation, counts of differentiating spermatogonia were reduced to a dose rate dependent level that remained essentially unchanged during the succeeding five months of irradiation; the production of A$\alpha$ spermatogonia (a population whose size depends on spermatogonial generations A$\alpha$-A$\alpha$) was not significantly affected by $1 \times 10^{-2}$ Gy d$^{-1}$ and average values for dose rates of $3 \times 10^{-2}$ Gy and $6 \times 10^{-2}$ Gy d$^{-1}$ were 60 and 30% of controls, respectively; testicular weight was also not significantly affected by $1 \times 10^{-2}$ Gy d$^{-1}$ but $3 \times 10^{-2}$ Gy d$^{-1}$ resulted in a final value of 73% of control and that for $6 \times 10^{-2}$ Gy d$^{-1}$ was 49% of control. Erickson concluded that a reduction of stem cell mitotic activity may be the principal effect of continuous low-level irradiation on spermatogenesis in the rat.

IV. TIMING OF OOCYTE MATURATION IN THE MOUSE AND ITS RELEVANCE TO RADIATION-INDUCED CELL KILLING AND MUTATIONAL SENSITIVITY

534. In the 1977 report, the preliminary results of Oakberg and Tyrell [O14] from their autoradiographic studies on the timing of oocyte development in the adult mouse were briefly discussed. Oakberg [O9] has now published these results and has also compared the nuclear morphology of arrested and stage 3 oocytes in the mouse, guinea pig and the human female. The results of Oakberg and those of some others not considered in the 1977 report are summarized in this section.

535. The importance of this problem derives from the fact that in specific-locus experiments with irradiated female mice, the mutation rate drops to zero in the progeny derived from conceptions that occurred after six weeks and it had not been possible so far to relate specific oocyte (and follicular) stages to specific post-irradiation litters. Pedersen's extensive analyses of follicular dynamics in the female mouse [P31] were based on $\gamma$-thymidine labelling of follicle cells; his results showed that it took 19 days for a stage 3b with about 20 cells to reach ovulation. Duration of stages 7 and 8, however, could not be measured by this technique. Other difficulties in using $\gamma$-thymidine labelling are that beta rays from the tritium kill follicle cells [B48]. Follicle cells have different cell cycle times, as a consequence of which undue weight may be given to rapidly dividing cells, thereby shortening the estimate of transit times.

536. In their earlier work using N-$\gamma$-thymidine-acetyl-D-glucosamine, Oakberg and Tyrell [O15] estimated that 35 days were required for maturation of stage 3b follicles. Although the problem of oocyte timing was not discussed by Haddad and Nagai [H46], the results of these authors with labelled L-fucose gave results similar to those of Oakberg and Tyrell, showing that the estimated time for maturation of 3b oocytes was longer than that in the work of Pedersen [P31].

537. In the work recently reported by Oakberg [O9] the question was asked whether the drop in mutation rate after six weeks might be attributed to the sampling of arrested stage 1 oocytes or whether it did occur after the initiation of follicular growth when species differences in chromosome morphology are minor. Four labelling experiments were performed, the first two with N-$\gamma$-acyetyl-D-glucosamine, the third with D-1-$\gamma$-glucosamine and the last one with L-1-$\gamma$-fucose. In experiment 1, the mice were given labelled acetyl-D-glucosamine (3 x 925 kBq at 9 h intervals) and killed at intervals ranging from 6 h to 25 days. In experiment 2, the females were irradiated with 50 R of x-rays (group 1) or given single intraperitoneal injection and 50 R of x-rays 24 h later (group 2) or the labelled compound alone (group 3). While some of the mice from these three groups were used for histological studies of the ovaries, some were pair-mated with 101 x C3H males. In experiment 3, the mice received D-1-$\gamma$-glucosamine (either in a single injection of 925 kBq and in three equal fractions spaced 9 h apart) and killed at intervals ranging from 24 h to 28 days after injection. Labelling with L-1-$\gamma$-fucose was achieved by two 925 kBq injections given 4 h apart and the animals were killed 1, 7, 14, 21 and 28 days after injection. The progressive appearance of unlabelled oocytes (unlabelled in zona pellucida) were used to compute the transit and the maturation time of the different stages.

538. The results show that:

(a) Of the three compounds tested, L-1-$\gamma$-fucose gave the best labelling with the lowest dosage;

(b) Approximately four weeks are required for stage 4a oocytes (with a second layer of follicle cells) to reach ovulation, thus confirming the earlier finding [O14]; the duration of stage 3b, however, is about two weeks instead of one week as suggested earlier;

(c) Adding these two estimates gives six weeks as the time interval from initiation of stage 3b to ovulation;

(d) Although there was some evidence for a reduction in the numbers of stage 3 and 4 oocytes in the N-
3H-acetyl-D-glucosamine-injected females, an extensive ten-week breeding test showed no effect on breeding behaviour or litter size of females given the labelled compound.

(e) There was no evidence that either 50 R or pregnancy had any effect on the progression of labelled follicles.

539. Thus, conceptions within six weeks after irradiation sample oocytes exposed in stages 3b–8 and litters conceived after six weeks are derived from stages 1–3a. The numbers of surviving stage 1 and stage 2 oocytes are so low (after irradiation) that no more than one or two litters are produced from these and at least one litter must be derived from stage 3a. The nuclear morphology of stages 3a and 3b are similar to one another and also to stage 3 of guinea pig and human oocytes and yet there are striking differences in mutational sensitivity of the 3a and 3b stage oocytes in the mouse; furthermore, the change in mutational sensitivity of the mouse oocyte with time after irradiation as shown by Russell [R38, R60] occurs in a cell with "typical" diploplite chromosome configuration, i.e., it does not parallel the change from the arrested diacety to the more characteristic diplotene of oocytes contained in a follicle with a single layer of cuboidal cells. These findings demonstrate that the degree of chromosome condensation does not appear to be a reliable criterion of oocyte sensitivity to either cell killing or mutation induction.

540. The high sensitivity to cell killing and the more diffuse (diacety) condition of the chromosomes of the arrested mouse oocyte compared to the radiation resistant "typical" diplotene of the arrested human oocyte has raised doubts concerning the relevance of genetic data in the mouse for the estimation of radiation hazards to the human female. The data of Caine and Lyon [C30] and of Cox and Lyon [C41], however, show that the guinea pig, where the oocyte is arrested in a condensed diplotene, with a high resistance to cell killing, also shows a low incidence of radiation-induced dominant lethals, just as the arrested diacety oocytes of the mouse and hamster.

541. There is therefore no consistent correlation, either negative or positive, between sensitivity to cell killing and the frequency of mutation observed [C30, R38]. The evidence so far suggests that low mutational sensitivity may be a general property of arrested mammalian oocytes (irrespective of nuclear configuration) but the number of species studied from this point of view is still small. Moreover, it should be pointed out that although the usual pattern for dominant lethal induction is one of high sensitivity of the maturing oocyte and low sensitivity of the immature one, Caine and Lyon [C30] found a somewhat higher frequency of post-implantation dominant lethals in the immature guinea-pig oocytes than in the mature, although the yield was still low.

542. As the authors point out, a considerable part of the high pre-implantation loss in first oestrus matings may have been due to oocyte damage rather than to dominant lethals. The evidence for dominant lethal induction was accompanied by a reduction in litter-size, lasting for many months after irradiation. The extent to which this reduction was the result of genetic damage is not yet clear. Caine and Lyon emphasize the need for further work on this, pointing out that "if genetic damage were detectable in guinea-pig oocytes several months after irradiation it would not be safe to conclude that such damage would not persist in human females". Therefore it would seem incorrect to assume that the mutational radiosensitivity of the immature human oocyte is necessarily as low as that of the immature mouse oocyte. Obviously, more data are needed.

V. SENSITIVITY OF MAMMALIAN FEMALE GERM CELLS TO KILLING BY IRRADIATION

543. The 1972 report of the Committee [U8] considered in some detail the sensitivity of oocytes to radiation-induced cell killing in different species of mammals. In this chapter, the main findings recorded in the above report will be first summarized before considering new data.

544. Female mammals are born with a finite number of oocytes formed already during embryonic development. These so-called primordial oocytes are surrounded by a single layer of follicular cells. With maturation, the oocytes grow and multi-layered follicles are formed. In young adults of both the rat and rhesus monkey, the number of growing oocytes amounts to about 10% of the total population, the remaining 90% being primordial follicles [B49].

545. In the oocytes, the sequence of nuclear changes comprising meiosis is arrested at the diplotene stage which lasts until the time of ovulation. The nuclear morphology of the arrested oocytes varies between species. Thus, a typical diplotene is characteristic of man, the rhesus monkey, the goat and the dog. A synizesis-like diploplite (chromosomes clumped into a dense knot) is characteristic of the guinea pig and a diffuse interphase-like diploplite (dicytate) is present in the mouse and a few related species of rodents such as the hamster, the deer mouse and the gerbil.

546. The chromosomes in the nucleus of the primordial oocytes in man and the rhesus monkey are of the so-called lampbrush type, similar in form to those of amphibia and other lower vertebrates. The oocytes in the growing follicles in all the species examined possess lampbrush-type chromosomes.

547. In humans, there is evidence [S81] that the oocyte chromosomes synthesize a large body of nucleolar material and that the synthesis is amplified during the diplotene stage. In pachytene, the oocyte nucleus shows 1 to 2 main nucleoli (connected with the satellites or secondary constrictions of D and G group chromosomes) and micronucleoli are rare. In diplotene however, the main nucleoli (2 to 3) become voluminous and numerous, micronucleoli appear and do not seem to be connected with the acrocentric chromosomes. They arise at the contact zones of the heterochromatic regions and differ from the nucleolar organizers on acrocentric chromosomes; they are connected in part to centromeric heterochromatin including various secondary constrictions.

548. In terms of radiation-induced killing of primordial oocytes, there are drastic differences in sensitivity between the different species. The response of the oocytes in the growing follicles, however, is not strikingly different. Information on these is summarized in Table 38.
549. A few papers (among them two reviews) dealing with the effects of irradiation on mammalian female germ cells have been published during the last few years [B48, B50, B51, H47]. Some of the information contained in the review papers [B50, B51], although not necessarily complete, covered a broader spectrum of mammalian species than was the case in the Committee's 1972 report. These data, however, strengthen the Committee's earlier conclusions, namely, that the sensitivity of the human oocyte to the killing effects of x-irradiation is more similar to that of the rhesus monkey than to that of rats or mice. In what follows, those aspects of radiosensitivity to killing not covered in the 1972 report will be presented with particular emphasis on the data from the rhesus monkey and man.

550. Oogonia in the ovaries of the rhesus monkeys and humans [B52, B53, B54] are more resistant than the corresponding cells in rodents. For example, Baker and Beaumont [B52] showed that the exposure of foetal rhesus monkeys aged 4–4.5 months to 350–600 R of x rays caused a 7% reduction in the population of oogonia; when the post-irradiation interval was extended to 10–14 days, the depletion of the germ cells was only 4%, reflecting the repopulation of oogonial cells by mitotic divisions. The proportion of oogonial killing was exposure-dependent; by increasing the exposure to 1000 R, the population was reduced to 43% of that in controls.

551. Baker and Neal [B54] showed that the sensitivity of oogonia in the ovaries of mice, rats and rhesus monkeys remains virtually the same irrespective of whether they are exposed to x-irradiation in organ cultures in vitro, or in vivo. Furthermore, they showed that exposure of 2000 to 4000 R eliminates only a proportion (80% during the sixth month of gestation) of the oogonia in human ovaries in vitro. This finding suggests that the human oogonia may be even more resistant than those of the rhesus monkey.

552. Data on the radiosensitivity of oocytes in foetal rhesus monkey are scanty. The available ones show that exposures between 200 and 400 R have no measurable effects on the population of oocytes [O16]. Baker and Beaumont [B52] found that none of the foetuses aged two months to full term (six months) was completely devoid of germ cells after 350–600 R of x rays and that a severe reduction of the number of oocytes occurred only after 1000 R or more. The most marked effect was found after exposure during the fifth month of gestation, a period associated with a high rate of spontaneous atresis and with the cessation of mitotic activity in oogonia [B52, B55].

553. Baker and Neal [B54] and Baker [B53] compared the structure of the irradiated and control human ovaries (from foetuses aged 2–7 months post-coitum) at various intervals after the onset of organ culture. Exposure to 2000–4000 R affected the germ cells at all stages in their development, although oogonia undergoing mitosis appeared to be the most sensitive. Quantitative studies of a foetal ovary from a six-month-old foetus showed that 4000 R x-irradiation caused a 65% reduction in the number of germ cells within 7 days of treatment. A similar result was obtained in foetal rhesus monkeys after exposure to 2000 R and confirms that human oogonia and oocytes are very radio-resistant.

554. The radiosensitivity of the oocytes of some juvenile and adult mammals has already been considered (Table 38). Most of the other mammalian species for which quantitative data are available suggest that they are intermediate between the mouse and the rhesus monkey. The LD_{90/10} for primordial oocytes in the guinea pig is about 500 R, although 15 000 R is required for 100% killing [I15], see also [O17]. This vast difference between the LD_{90} and LD_{900} values is presumably due to the fact that there are two distinct populations of primordial oocytes: one, a "large" type, with appearance similar to those of other species such as the monkey and man and the other, a "contracted" type, in which the chromosomes appear to be condensed toward the centre of the nucleus. "Contracted" oocytes are radiosensitive: they are either killed by exposure to x rays or are transformed into the larger types [I15].

555. Only qualitative data are available for the rabbit and the hamster. Oocytes in these species seem to have radiosensitivities comparable to those in the rat [B56, M50]. Thus, doses of less than 7 Gy destroy the majority of primordial follicles and also a proportion of those that have completed their growth phase. Females of species that have relatively long foetal or post-natal development, in contrast to those of rapidly developing species (such as rodents), are not sterilized by acute radiation exposure. The reason for this is owing to the greater asynchrony of oocyte stages in the former species. Even in the slowly developing species, however, continuous or fractionated exposure has revealed a highly sensitive developmental stage of limited duration. This has been found to be the case for bonnet monkeys (Macaca radiata) [A31], squirrel monkeys (Saimiri sciureus) [D25], and juvenile dogs [A32]. species in which the arrested oocyte is resistant to radiation-induced cell death. Continuous exposure of foetal squirrel monkeys to tritiated water indicated a sensitivity of developing oocytes even higher than that observed in the mouse. The finding that primates, as well as mice, have oocyte stages that are vulnerable to radiation suggests that the human oocyte may also pass through highly sensitive stages in development. The general pattern of sensitivity of oocytes to radiation among mammalian species may be more similar than is commonly thought.

556. Preovulatory maturation of oocytes within Graafian follicles commences with the onset of the so-called "LH surge" (Luteal hormone) which occurs, for example, about 13 hours before ovulation in the mouse and 36 hours before ovulation in man [B50]. Thereafter, radiosensitivity varies according to the stage of meiosis attained by the oocyte and increases by a factor of 10 as the cell passes from the dictyate stage to metaphase I in the mouse [M51].

557. Hobson and Baker [H47] studied the effect of ovarian x-irradiation in the rhesus monkey on menstrual cycle length, duration of menstrual bleeding, excretion of gonadotrophin in the urine, concentration of gonadotrophin in the pituitary gland, ovarian histology and breeding performance. It was found that at exposure below 4000 R, there was no significant effect on any of the above parameters measured. However, exposures of between 400 R and 6000 R rapidly induced amenorrhoea in most animals, but no increased excretion of gonadotrophin was observed. The reproductive capacity of the animals used in the study was poor. No births occurred before irradiation and only three confirmed pregnancies thereafter.

558. Baker and McLaren [B48] conducted experiments to measure the cell-killing effects of ^3H-thymidine on the developing oocytes of mice using
a multiple injection method similar to that in the work of Callebaut [42]. Pregnant Q strain mice received seven injections of ³H-thymidine (148, 1480 kBq and 14.8 MBq per injection) between the 13th and 16th day of pregnancy at about 12 h intervals. The animals were either killed on the 17th day of pregnancy and the foetal gonads removed for analysis or they were allowed to give birth and the ovaries of the progeny were studied.

559. It was found that:
(a) The ovaries of the foetuses on the 17th day of gestation and on the day of birth contained oocytes which were predominantly at the zygote or pachytene stage of prophase I and the majority of these cells were labelled;
(b) The highest intensity of silver grains was over the oocytes;
(c) Oocytes from experimental mice aged up to one month appeared normal, although smaller than in the controls; the proportion of the oocytes undergoing degeneration was higher than in the oocytes of control mice and in the treated ovaries, the atretic oocytes being more heavily labelled;
(d) In 5-months old experimental mice, the primordial follicles were more numerous in the appearance in ovaries from the low dose group (148 kBq); in the high dose groups, however, there was an increase in the proportion of grossly abnormal follicles and some oocytes were devoid of oocytes;
(e) There was a reduction in the total number of oocytes in the ovaries of experimental mice and this was proportional to the dose of the isotope used and affected primordial follicles more than multilayered follicles; by 5 months of age, the numbers of oocytes in the 1480 kBq and 148 kBq groups were reduced to about 1% and 50% of the control values, respectively;
(f) At 13 months of age, ovaries from mice in the 14.8 MBq group were devoid of oocytes and corpora lutea, while in the 1480 kBq group some corpora lutea remained, but virtually no oocytes. These results permitted the authors to conclude that the mouse oocytes are highly sensitive to internal beta-radiation from ³H-thymidine incorporated during embryonic life.

VI. SOMATIC CELL GENETICS

560. Only a limited amount of data has accumulated since the publication of the 1977 report on mutation induction in somatic cells by ionizing radiation. The papers concerned and some others dealing with other kinds of radiations will be briefly reviewed in the following paragraphs.

A. MUTATION INDUCTION AT THE HG-PRT LOCUS

1. Chinese hamster ovary (CHO) cells

561. It is known that several factors, such as the analogue used, its concentration, cell-seeding density, time of addition of the analogue to the mutagen-treated cells (expression time), the source of serum used in the selective medium, interclone metabolic co-operation etc., affect the recovery and type of mutants obtained. Jostes et al. [11] investigated the role of de-amination of 8-azaguanine (8-AG) to 8-azaxanthine (8-AX) by serum under conditions where cell density and expression time were controlled. It was found that the reduction in toxicity of 8-AG which results from its deamination to the non-toxic product 8-AX leads to the selection of many mutants which are resistant only to drug concentrations lower than that added to the selecting medium. That is, at lower concentrations one may be selecting preferentially for mutants with partial enzyme activity or for non-mutant cells. In fact, an analysis of mutant clones selected under conditions where drug toxicity was maintained revealed that most of the mutants having partial enzyme activity were eliminated. The elimination of partial mutants agreed well with the approximately 2-fold reduction in frequencies of spontaneous and x-ray induced mutants when drug toxicity was maintained by single 30 µg AG/ml and by changing the drug medium once.

562. Burki [80] found that when exponentially growing CHO cells are exposed to 50-kVp x rays, the survival curve shows a shoulder and the induction of 6-TG₈ mutations is curvilinear with dose (2-10 Gy), responses similar to those reported by other investigators for exposures of CHO and V-79 cells to more energetic x rays. When the cells are synchronized without the use of drugs, the appearance in ovaries from the low dose group (148 kBq); in the high dose groups, however, there was an increase in the proportion of grossly abnormal follicles and some oocytes were devoid of oocytes;
those induced by the two single doses, suggesting that “premuntational” lesions were repaired.

565. In the study of Cleaver [C43] cultured CHO cells were labelled with 6-3H-thymidine or 5-methyl-3H-thymidine, frozen and allowed to accumulate damage from 3H decays for varying periods of time. Two series of experiments were carried out with each of these. At the end of various “dose accumulation periods”, the cells were thawed to 37°C and used for the determination of 6-TGR mutations. Appropriate controls were maintained. In parallel experiments, mutation induction by UV (up to 24 J/m²) and x rays (up to 10 Gy) was also studied.

566. The results showed that, in the range of doses employed, the dose-response curves were linear for all three experiments. 3H in the 6 position produced 2–3 times more 6-TGR mutants than 3H in the 5-methyl position, indicating that a local effect associated with transmutations at the 6 position in the DNA produces mutations more efficiently than the emitted beta particle does. No difference between 3H decays at the 6 position and the 5-methyl position was observed when DNA damage in the form of single-strand breaks was measured. In another study, Cleaver [C44] examined whether or not x-irradiation would induce an error-prone repair process that would increase the frequency of 6-TGR mutants in CHO cells. The cells were x-irradiated at times from 0 to 17 h before being irradiated with UV. Only one x ray dose (3 Gy) and one UV dose (13 J/m²) were used. No synergism however, was observed.

2. Chinese hamster V-79 cells

567. Thacker, Stretch and Stephens [T23] examined the induction of 6-TGR mutants in the V-79 Chinese hamster cells after gamma irradiation. After irradiation, the cells were grown in non-selective medium for different time intervals before respreading into medium containing 0.5 to 0.7 μg/ml 6-TG; in some experiments, colonies arising in 6-TG medium were counter-selected in medium containing the glutamine-analogue azaserine, which distinguishes mutants with very little HG-PRT activity. Only these mutants were found to be increased in frequency by irradiation, the maximum measured frequencies occurring in cells respread after two days of growth in non-selective medium. The results showed that over the range from 1–8 Gy the dose-effect relationship for induced mutations per viable cell was non-linear (induction rates of 5 × 10⁻⁸ per 10⁻² Gy to 3 × 10⁻⁷ per 10⁻² Gy). However, a plot of the induced mutation frequency against log surviving fraction gave an approximately linear relationship. The same linear relationship holds for recently published data on human and mouse cell cultures, so that all three mammalian cell types exhibit the same fixed probability of mutation induction relative to the extent of inactivation caused by ionizing radiation (about 4 × 10⁻³ mutants per lethal event) (see also [T33]).

568. Asquith [A25] studied the mutagenic and lethal effects of single and fractionated doses of gamma irradiation in the same cell line. The induction of 8-AGR mutants was scored. The results showed that:

(a) The dose-effect curve after single doses (0.5 to 10 Gy) was non-linear;
(b) The lethal and mutagenic effects of fractionated doses were always less, relative to those after single exposures;
(c) When the cells were exposed to graded single doses of up to 6 fractions (each of about 3.5 Gy), the rate for fractionated doses always was lower than that for the corresponding single doses; and
(d) When a total dose of 8.34 Gy was administered either singly or in 2, 3, 4, 6 or 12 fractions of equal size, there was an increase in survival with increasing number of fractions paralleled by a concomitant decrease in mutation frequency, such that there appeared to be a constant relationship between mutation and survival.

569. Thacker et al. [T24] studied mutation to 6-TG resistance in V-79 Chinese hamster cells after irradiation with accelerated helium, boron or nitrogen ions covering a LET range from 28 to 470 keV µm⁻¹. It was found that for all radiation qualities, a dose-dependent increase in mutation frequency was obtained for doses giving surviving fractions greater than about 0.20. The effectiveness per unit dose for both inactivation and mutation induction increased with increasing LET of the radiation to a maximum in the range of 90–200 keV µm⁻¹. However, the maximum mutagenic effectiveness (relative to gamma rays) was about 2 or more times that for inactivation.

570. Unique RBE values cannot be given for these data because of the differences in the shapes of the dose-effect curves at different LETs. The linear coefficient taken from the quadratic fit to each set of data gives one estimate of RBE which emphasizes the difference in effectiveness at low doses. This “initial slope RBE” for helium ions (for mutation induction) range from 2.9 to 17 in the range of LETs from 20 to 90 keV µm⁻¹; for boron ions, at both LETs tested (110 and 200 keV µm⁻¹) the RBE values are the same, while for nitrogen ions this is 14.

571. In a subsequent paper, Goodhead et al. [G43] used carbon-K characteristic ultrasoft x-rays of photon energy 0.278 keV. These x rays produce electrons of “range” ≤ 7 nm which is an order of magnitude smaller than those for aluminium x rays and is only about three times the diameter of the DNA double helix. The effective ranges are less than 7 nm owing to the very tortuous path of such slow electrons; each electron produces a total of only about 34 ionizations and these are not all along a clearly defined path. The dose distribution through the cell is highly non-uniform being 100% on the entrance surface and decreasing exponentially with depth in the cell. Under the assumption that the targets are uniformly distributed within the cell of uniform thickness 7 μm, the average dose to the targets is 24% of the dose at the entrance surface. Despite the very low energy and short track length, the authors found that the above ultrasoft x-irradiation caused inactivation and induced 6-TGR mutants. With certain assumptions about the position of the sensitive sites within the cells, it was concluded that carbon x rays are more effective than gamma rays and are probably at least as effective as long tracks of helium ions of similar LET. These data extend the conclusions previously drawn from the observed effectiveness of aluminium x rays [C67, G44] regarding the sizes of the sub-cellular targets involved in inactivation and mutation induction. They imply that the sensitive sites are smaller than about 7 nm and that highly localized energy depositions consisting of ≤ 14 ionizations are sufficient to produce biological effects.

572. The increasing use of photochemotherapy, involving combined treatments with 8-methoxypsoralen
(8-MOP) and long-wave UV light (UVA: emission range: 305–450 nm with a maximum at 355 nm) for treatment of psoriasis in man prompted Burger and Simons to study the effects of similar treatments (PUVA) on mutation induction and cell-killing in V-79 Chinese hamster cells [185] and in diploid human fibroblasts [186]. This study is considered later in this chapter. Treatment with 8-MOP alone (50 μg/ml, 4 h) or UVA alone (9000 J/m²) did not cause any significant induction of 6-TG mutants. Combined treatment (PUVA) induced both cell-killing and mutations. This was also observed under conditions approaching patient treatment (PUVA) with respect to the concentration of 8-MOP in the skin and the amount of UVA received by the epidermal cells. A simple relationship proved to be applicable for mutation induction under different conditions: 5.5 10⁻⁴ per J/m² per μg of 8-MOP.

3. Mouse lymphoma (L 5178Y) cells

573. Nakamura and Okada [118] investigated the gamma-ray (¹³⁷Cs) induction of 6-TG⁻ mutations in L5178Y cells at 0.5 Gy min⁻¹ and 8 10⁻³ Gy min⁻¹. In addition, they examined cell killing, the relationship between mutation frequencies and variations in dose rate in the range of 0.5 to 8 Gy min⁻¹ at a dose of 2 Gy, the effects of temperature (± 0°C versus 37°C during irradiation at 0.5 or 8 10⁻³ Gy min⁻¹; 2 Gy) and the effects of adding dimethyl sulphoxide (DMSO) at various concentrations to the medium containing the cells during irradiation.

574. For cell killing from 2 to 8 Gy the dose-response curve was sigmoidal at high dose rate and exponential at low dose rate. In the same dose range, the frequency of 6-TG⁻ mutations increased faster than linearly with high dose rate irradiation, but was nearly linear at low dose rate. At a dose of 2 Gy, the mutation frequency continued to decline (to about one-half of that after high dose rate irradiation) with a decrease in dose rate until the latter reached about 3.3 10⁻² Gy min⁻¹, below which there was no further reduction. At 0°C, the frequencies of mutations were nearly the same at both the high (0.5 Gy min⁻¹) and low (8 10⁻³ Gy min⁻¹) dose rates, whereas at 37°C the frequency at the lower dose rate was less than one-half of that at the higher one. DMSO exhibited a radioprotective effect both with respect to survival and to mutation induction; the decrease in mutation frequencies became more pronounced with increasing DMSO concentration following irradiation at 0.5 Gy min⁻¹ but were not measurably affected by irradiation at the low dose rate.

4. Human diploid fibroblasts

575. In the experiments of de Ruijter and Simons [61], human diploid fibroblasts were x-irradiated (100, 200 and 300 R) in suspension and mutation induction at the HG-PRT locus was studied using the 8-AG or 6-TG selection systems; in addition, the length of the expression time for these mutants was ascertained by seeding the cells for selection at 0, 3, 6, 10 and 14 days after x-irradiation. The results showed that:

(a) Direct expression of at least a proportion of the mutants occurred on day 0 followed by an increase in mutant frequency over the entire culture period; the latter, however, was found to be entirely due to an increase in spontaneous mutations, suggesting that for human diploid fibroblasts, culturing after treatment is not necessary for the expression of HG-PRT deficient mutants.

(b) In practice, however, a culture period is needed as the number of clone-forming cells that can be seeded without culture after treatment is very low, and the number of mutants that can be selected is consequently small, even in large-scale experiments.⁰⁸

(c) The exposure-frequency relationship does not appear to deviate from linearity; the mean mutation rate is 2.1 10⁻⁷ per R which, in the exposure range used, is about twice that obtained in rodent cells.

576. Cox and Masson [45] studied the induction of cell killing and mutations to 6-TG⁻ in cultured human diploid lung fibroblasts (HF 19 strain) after exposure to ionizing radiations with LET in the range from 20 to 470 keV μm⁻¹. It was found that, for all radiations studied, the dose-response curves were exponential (range: up to 2.5 Gy). Helium ions of increasing average LET showed increasing effectiveness in the order: He(20) < He(28) < He(50) < He(70) < He(90). The D₅₀ values decreased from 0.92 Gy at 20 keV μm⁻¹ to 0.32 Gy at 90 keV μm⁻¹. Correspondingly, the RBE values increased from 1.4 to 4.0. Boron ions of increasing average LET showed decreasing effectiveness in the order B (110) > B (160) > B (200). The D₅₀ values increased from 0.34 Gy at 110 keV μm⁻¹ to 0.5 Gy at 200 keV μm⁻¹. The RBE values correspondingly decreased from 3.7 to 2.5. Nitrogen ions of 470 keV μm⁻¹ were less effective than B ions of 200 keV μm⁻¹; the D₅₀ was 0.73 Gy and the RBE, 1.7. In view of the fact that the survival curves were exponential, the RBE values for inactivation were not dose-dependent; the RBE maximum lies between LETs of 90 and 110 keV μm⁻¹ and is of the order of about 4.

577. The dose-response relationships for mutation induction were approximately linear for all radiation types. Helium ions of increasing average LET showed increasing mutagenic effectiveness in the same order as that for cell killing; the induced mutation rates increased from 3.9 10⁻⁵ Gy⁻¹ at 20 keV μm⁻¹ to 22 10⁻⁵ Gy⁻¹ at 90 keV μm⁻¹. Correspondingly the RBEs increased from 1.3 to 7.1. Boron ions of increasing average LET showed similar mutagenic effectiveness; the induced mutation rates were between 17.8 and 20.7 10⁻⁵ per Gy. The RBE values were between 5.7 and 6.7. Nitrogen ions of 470 keV μm⁻¹ were considerably less effective than boron ions, the induced mutation rate being 8.5 10⁻⁵ Gy⁻¹ and the RBE 2.8.

578. The mutation data suggest that the RBE-LET relationship has a humped form similar to that for cell inactivation. However, while the RBE values for inactivation and mutation induction differed only slightly for radiations in the LET range between 20 and 50 keV μm⁻¹, at higher LET these values for mutation were about twice those of the corresponding RBEs for inactivation. The RBE maximum for mutation induction is in the range 90–200 keV μm⁻¹. Although this range is similar to that obtained for the induction

⁰⁸ It is instructive to recall that in the work of Cox and Masson [71] discussed in the 1977 report, it was demonstrated that, with respect to the induction and selection of 6-TG⁻ mutants, the maximal yield was observed when the cells surviving the irradiation had completed 3–4 doublings (6–7 days of growth) in a non-selective medium.
of 6-TGR mutations in V-79 Chinese hamster cells [T24], the comparison of RBE is complicated by the fact that the hamster cell RBEs are not unique values.

579. In experiments with aluminium soft x rays and carbon soft x rays, Cox et al. [C97] and Goodhead et al. [G91] demonstrated that these irradiations are capable of causing cell inactivation and inducing 6-TGR mutations in human cells. The general conclusions are similar to those arrived at using V-79 cells.

580. Burger and Simons [B58] investigated cell killing and induction of 6-TGR mutations in dividing and non-dividing (i.e., under liquid-holding conditions) human skin fibroblasts derived from a healthy boy) from treatment with 8-MOP and long-wave UV (UVA). Two 8-MOP concentrations (0.25 and 10 μg/ml) and a range of UVA doses (up to 100 J/m² in combination with 10 μg/ml 8-MOP and up to 100 J/m² in combination with 0.25 μg/ml 8-MOP) were used. Under liquid-holding conditions, both the amount of cell killing and the frequency of induced mutations are lower than for dividing cells under the same 8-MOP/UVA conditions; the effect of liquid-holding on cell survival is less pronounced (reduction by 40%) than on mutations (reduction by 80%). The number of mutations induced per unit dose (= μg 8-MOP/ml per J/m²) in dividing and non-dividing human fibroblasts is 3.06 × 10⁻⁶ and 0.25 × 10⁻⁶ for 1.5 × 10⁻⁴ M 8-MOP and UVA dose range up to 100 J/m² and for 0.25 μg/ml 8-MOP and UVA range up to 3500 J/m² this finding being qualitatively similar to that obtained with V-79 cells [B57]. These data thus suggest that there is a linear relationship between mutant yield and the product of 8-MOP concentration times UVA dose. The cytotoxic effect of PUVA treatment, however, is dependent on the actual concentration of 8-MOP and UVA dose and this result is also in agreement with the results with V-79 cells. Calculations show that, for instance, at 50 and 10% survival levels, the decrease of 8-MOP concentration by a factor of 40 (from 10 to 0.25 μg/ml) can be compensated by an increase of UVA exposure by a factor of 10. This suggests that the increase in UVA exposure needed does not bear a simple proportionality to the decrease of 8-MOP concentration with the combined treatment.

581. Burger and Simons have used the above data to calculate the risk of mutation induction in epidermal cells for persons receiving PUVA therapy of psoriasis and have shown that the concentration of 8-MOP in the skin is similar to that of skin fibroblasts; that the concentration of 8-MOP in the skin during a normal PUVA treatment is 0.25 μg/ml; that about 30% of the dose of UVA reaches the basal layer; and that about 10% of the epidermal cells are dividing and 90% are in G0. In clinical studies, Burger et al. [B55] used a starting dose of 18 000 J/m² for fair-skinned individuals which, for the basal layer of the epidermis would mean a dose of 5400 J/m². The weighted average frequency of mutations per PUVA session then becomes

\[ 5400 \times 0.25 \times 3.3 \times 10^{-8} \times 0.10 \text{ (for dividing cells)} + 5400 \times 0.25 \times 0.6 \times 10^{-8} \times 0.90 = 1.17 \times 10^{-5} \]

Patients given 36 maintenance treatments a year for 30 years will accumulate 30 x 36 x 1.17 x 10⁻⁵ = 1.26 x 10⁻⁴ per cell. This figure might be of some relevance in the context of assessing potential risk of cancer induction (on a mutation model) for patients given PUVA therapy if such therapy can be shown to have a carcinogenic effect.

5. Human peripheral blood lymphocytes

582. In 1977, Strauss and Albertini [S120] reported on an autoradiographic method to quantitatively detect 6-TGR variants in human peripheral blood lymphocytes. In subsequent papers, Albertini [A30] and Strauss and Albertini [S121] discussed in detail the experimental procedures and the results obtained in studies with lymphocytes of normal individuals, from cancer patients treated with cytostatic chemicals or x-radiation therapy and from Lesch-Nyhan syndrome (LN) patients.

583. Albertini [A30] and Strauss [S120, S121] made use of the cytotoxicity of 6-TG to human lymphocytes possessing normal HG-PRT levels to distinguish and select for viable 6-TGR variants. In autoradiographic studies, such variants were shown to undergo DNA synthesis (incorporation of 3H-labelled thymidine) and survive in the presence of normally toxic levels of 6-TG, following PHA stimulation. Experiments with artificial mixtures of lymphocytes from LN patients and normal individuals showed that the LN cells were virtually all detectable even when present in low frequency (10⁻⁵). 6-TGR lymphocytes were found in healthy normal individuals at median frequencies of 1 in 10⁴ and 1.1 in 10⁻⁴ while determined at 2.10⁻³ and 2.10⁻⁴ M concentration of the select agent. Their frequencies were not found to be age-related. The distribution of the frequencies of 6-TGR variants in cancer patients (treated with chemotherapeutics) differed from that for normal controls in that more than half of the patients had the variants in frequencies higher than the highest seen in controls. Such an increase in variant frequency over control levels was also noted in lymphocytes from radiotherapy patients [A30].

584. In their studies, Strauss and Albertini also found that lymphocytes from even LN patients which were resistant to 6-TG at concentrations 10 000 times over those which inhibit normal cells, were not absolutely resistant; thus a marked difference in the apparent frequency of 6-TGR cells was seen when LN cells were tested at 2 10⁻⁴ M and 2 10⁻³ M: only the cells at the higher concentration were able to incorporate 3H-thymidine. Secondly, in the chemotherapeutically exposed cancer patients, the 6-TGR fraction of cells was different at 2 10⁻⁴ M than at 2 10⁻³ M, suggesting that many of the 6-TGR lymphocytes had properties similar to LN cells. Thirdly, in some of these cancer patients, the levels of 6-TGR cells were higher even before chemotherapy with further rises either after the initiation of chemotherapy, or after its re-initiation after a non-treatment interval. Fourthly, PUVA treatment for psoriasis also leads to elevated variant frequencies. Finally, the authors have evidence to suggest that some cells that synthesize DNA in vitro under the conditions used for selection may not be mutants but rather may be phenocopies.

585. Considering all the data, Strauss and Albertini suggest that at least some of the variants isolated are somatic cell mutants and that the system in its simplest application may be valuable as a retrospective monitoring system for unknown environmental contaminants, for combination of agents, etc.

586. Evans and Vijayalaxmi [E23] have now collected data on the x-ray and mitomycin-C induction of 8-AGR variants in vitro studies with human lymphocytes. In parallel experiments, determinations of chromosome
aberration frequencies (after x rays) and of SCE frequencies (after MMC) were also made.

587. The reason for using 8-AG for variant selection has been stated as follows by the authors: "We were strongly influenced by the fact that 6-TG is incorporated into DNA and that inhibition of DNA synthesis by excess thymidine protects against the toxicity of 6-TG, but not 8-AG. In contrast, 8-AG is incorporated into RNA and inhibition of RNA synthesis protects against the killing effects of 8-AG, but not of 6-TG. The short-term culture of lymphocytes in vitro is inappropriate for selecting for resistance to cell killing post-DNA synthesis, and since the blast formation of lymphocytes by PHA necessitates a considerable amount of RNA synthesis, we elected to use 8-AG (2 x 10^3 M) in preference to 6-TG in all the experiments." [E23].

588. The main results are that the incidence of 8-AGR variants varied from 0.80 x 10^{-4} to 3.99 x 10^{-4} in the blood samples from 26 donors whose ages ranged from 16 to 82; although there was considerable scatter, there was nevertheless a clear age dependence (the frequencies increased with age); there was no sex-difference in the frequency of these spontaneous variants; significant increases in 8-AGR variant lymphocytes occurred following exposure to quite low doses of x-rays and the dose-response over the range 0–2 Gy is curvilinear; the data give a good fit to a quadratic equation with a negligible linear term or to a power-law function with n equal to 2.1. Assuming that n = 2, then the linear term is equal to (6.90 ± 0.11) x 10^{-4} and the quadratic term, (6.81 ± 0.20) x 10^{-4}; the kinetics of dose-response for the x-ray induction of 8-AGR variants is similar to that for x-ray induction of chromosome aberrations; with MMC (treatment times of 1, 5 or 10 h in the concentration range 10^{-9} to 10^{-3} M) the frequencies of the variants increased with MMC concentration to the first-order dose-response being of the form y = a + bD where y = variant frequency, a = control frequency of (8.38 ± 0.37) x 10^{-4} and D = MMC concentration. The values of b (for treatment durations of 1, 5 and 10 h) are respectively, 0.83, 0.92 and 1.06; the maximum increase in 8-AGR variants is around 12-fold at the highest MMC concentration, but significantly increased frequencies are evident following exposure to low doses in 8-AGR variant lymphocytes. The data on SCEs show that there are induced at very much higher frequencies relative to the 8-AGR variants per cell, but the shape of the dose-response curves for these two end-points are very similar. The authors have suggested that x-ray induced 8-AGR cells may be true mutants (the spontaneous variants are probably deletions involving the terminal region of the X-chromosome containing the locus for HG-PRT) and that on the basis of their evidence, for every 8-AGR event in a cell population, there are around 10 visible chromosome breaks in an active X-chromosome. The MMC-induced 8-AGR variants are considered to represent "cells in which large MMC-guanine adducts have prevented normal transcription of the HG-PRT locus to give functional HG-PRT."
evidence for an interaction between cellular radiation
damage and ouabain (irradiated wild-type cells were
more rapidly inactivated by ouabain than unirradiated
cells) but this damage seems insufficient to account for
the inability to detect OUA\textsuperscript{R} mutations after ionizing
radiation.

592. Chang et al. [C47] irradiated Chinese hamster
V-79 cells with an unequally fractionated UV dose
(UV\textsubscript{t}, 32/jm\textsuperscript{2} + 171/jm\textsuperscript{2}) separated by 4.6 or 12 h, with
or without cycloheximide or caffeine present during the
interval between the fractions, for part or whole of the
period. Among others, the effects of these different
regimes on post-replication repair, survival and
mutation induction to OUA\textsuperscript{R} were studied. The data on post-replication repair (collected using alkaline sucrose
density gradient methods) show that irradiating cells
with an initial small dose of UV, 4–6 h before a larger
UV dose, significantly increases the rate by which small
DNA, found immediately after irradiation is converted
into high molecular weight DNA; the presence of UV-
damaged DNA for at least 1.5 h before UV\textsubscript{t} irradiation
appears to be sufficient to enhance the rate of post-
replication repair. This enhancement is inhibited by
cycloheximide treatment during the interval between
fractions; a similar inhibitory effect was observed with
caffeine.

593. The data also demonstrate that with fractionated
UV irradiation, the colony-forming ability was higher
relative to the unfractionated dose; however, the
mutation frequencies were either reduced or were not
affected. The results from cycloheximide treatments
gave two different kinds of effects, depending on the
protocol used; a 4 h treatment immediately before the
second irradiation increased survival but reduced
mutation frequencies whereas a 6 h treatment (or
treatment long before UV\textsubscript{t}) tended to reduce survival
and increase the mutation frequency. While the
mechanisms for the two different effects of cyclohex-
imide treatment are not clear, the authors speculate that
this chemical may have dual effects, one promoting the
action of an error-prone system (i.e., causing the
observed increase in mutation frequency) and the other
an error-free system (causing the observed reduction in
mutation frequency). With respect to caffeine, the
results show that caffeine pre-treatment or treatment
between the fractionated UV doses always increases the
mutation frequency. Since caffeine does not effect excision repair in Chinese hamster cells, these data indicate that caffeine pre-treatment may inhibit an
error-free post-replication repair system, while allowing
error-prone repair to occur.

594. In Cleaver's studies on the induction of TG-
resistant mutants in CHO cells by radioactive thymidine discussed earlier in this chapter OUA\textsuperscript{R}
mutations were also sought for, but none found. His
other experiments [C44] on synergism between the
effects of UV and x rays for mutation induction to
OUA\textsuperscript{R} provided no evidence for such an effect.

D. RESISTANCE TO METHOTREXATE (MTX\textsuperscript{R})

595. In the work of Nakamura and Okada on the
gamma-ray induction of 6-TG\textsuperscript{R} mutations in L5178Y
cells the induction of MTX\textsuperscript{R} mutations was also
studied. It was found that the dose-response curve for
these mutations was linear, irrespective of the mode of
delivery (high versus low dose rate) of the irradiation
although the rate of increase with increasing dose was
less after low dose rate irradiation. As in the case of 6-
TG\textsuperscript{R} mutations, presence of DMSO in the medium at
the time of high dose rate irradiation afforded a radio-
protective effect, this effect increasing with increasing
DMSO concentration.

E. NATURE OF RADIATION-INDUCED
MUTATIONS IN SOMATIC CELLS

596. In 1978, Cox and Masson [C46] presented
evidence that gross structural changes involving the X
chromosome constitute the genetic basis of a significant
proportion of radiation-induced 6-TG\textsuperscript{R} mutations in
human fibroblasts. Gene mapping experiments have
assigned the HG-PRT locus in humans to the terminal
region of the long arm (q27) of the X chromosome
[P41]. Cox and Masson determined the pattern
disruption points (PDPs) for 23 6-TG\textsuperscript{R} mutants induced
by 250 kV x rays, carbon ultrasonic x rays, helium ions of
90 keV/\mu m, alpha particles from plutonium (~ 140
keV/\mu m) and nitrogen ions of 470 keV/\mu m. These
mutants were selected by resistance to 3 \mu g/ml 6-TG,
seven days after irradiation at dose levels that gave
surviving fractions of 0.2 (about 2 Gy of x rays). The
hypothesis was that, if radiation-induced mutations to
6-TG\textsuperscript{R} arise as a consequence of structural changes of the
X chromosome, a proportion of the more extreme
changes should be detectable cytologically, using
banding techniques.

597. It was found that aberrations (exchanges and
deletions) consistent with the mapped position of the
HG-PRT locus were apparent in the X chromosomes of
up to 40% of TG\textsuperscript{R} mutants. In the case of exchange
aberrations, the independence of the mutants described
was clear, since all the exchanges were different. The
majority of mutants with X chromosome deletions were
detected in a single experiment using nitrogen ions and
it is possible that two examples of del(Xq27) were
repeats of a single clone. With a single exception (an
Xp exchange probably not associated with the mutant
phenotype) all PDPs occurred in the q arm of the X
chromosome.

598. Analysis of 20 spontaneous mutant karyotypes
failed to reveal any structural abnormalities in the X
chromosome. Since a direct association between
induced TG resistance and X-chromosomal aberrations
would require that such aberrations in non-mutant cells
surviving the radiation dose should be rare, 25 non-
mutant viable clones were also examined cytologically
(alpha particles from plutonium were used); no aberr-
ations were found.

599. The conclusion that structural changes of the X
chromosome are associated with the loss of HG-PRT
function raises the question of whether the functions of
other genes located on the long arm of the X are also
affected. As a preliminary test of this possibility, the
authors examined the G-6-PD activity using semi-
quantitative histochemical staining techniques. As
judged by the staining intensity, the variations in
G-6-PD activity among the alpha-particle induced TG-
resistant mutants were considerably larger than those
observed among non-mutant surviving clones, with up
to 5% of the mutant clones showing very low activity
and a similar number showing hyperactivity. In electrophoretic enzyme assays, two karyotypically defined Xq
exchange mutants were completely devoid of HG-PRT
activity but were not obviously deficient or super-active
in G-6-PD activity. However, because almost all mutant
clones showing atypical G-6-PD activity in histoch- 
chemical assays showed very poor growth, it has not 
been possible to carry out truly representative 
karyotype and electrophoretic analyses and hence the 
question of multiple gene effects in radiation-induced 
TG mutants still remains to be answered.

600. Brown and Thacker [B75] stress that the methods of 
human fibroblast HG-PRT mutant selection and the 
limited life span of mutant clones preclude precise 
estimates of the proportion of radiation-induced 
mutants with recognizable X-chromosome aberrations. 
The 40% figure quoted in the paper of Cox et al. [C46] 
was an average figure for a variety of different qualities 
of ionizing radiation and these preliminary experi-
ments, no attempt was made to avoid the problems of 
mutant fitness and consequently there was considerable 
experimental variation in the proportion of chromo-
osomal mutants detected. However, Brown et al. 
generally observed that 250-kV x rays generated a lower 
proportion (5–25%) of such mutants than radiations of 
1000-LET (20–60%). Since the proportion of such 
mutants observed was influenced by the duration of the 
post-irradiation growth (for mutant expression) and 
many mutant clones showed such poor growth that they 
could not be characterized, these authors are dis-
inclined to put too much emphasis on these figures; 
interest in a good case for the existence of the 
ration-induced HG-PRT mutants can be made on 
the basis of biochemical evidence. Such evidence has now been obtained.

601. The evidence comes from HG-PRT mutants in 
V-79 Chinese hamster cells [B75]. Each of these mutants 
was independently isolated (the experimental design 
ensured this) although inevitably, some spontaneous 
mutants will be included in those isolated after irrad-
iation. The analysis pertains to a total of 50 mutants 
isolated after a 5 Gy gamma-ray exposure (6-TGR 
mutants) and to similar numbers of spontaneous and 
EMS-induced mutants. All of these were examined in 
cell-free extracts for HG-PRT activity. Approximately 
10% (5/48) of radiation-induced mutants showed 
measurable (i.e., more than 1% of the activity of 
wild-type cells) HG-PRT activity while 50% (23/46) of 
spontaneous mutants showed such activity. Since the 
radiation treatment gives only a 5-fold increase in 
mutant frequency over that found spontaneously at this 
dose, it is good case that all those mutants with 
measurable activity found after irradiation are of 
spontaneous origin.

602. Other evidence to support this idea is that the 
frequency of mutants with measurable HG-PRT 
activity does not increase with radiation dose, as was 
demonstrated earlier by Thacker et al. [T30]. In 
addition, out of a small number (15) of independently-
isolated mutants induced by alpha particles at a dose 
giving a 14-fold increase in mutant frequency, none 
showed measurable HG-PRT activity.

603. Brown et al. [B75] checked for the presence of 
HG-PRT protein in those mutants which showed no 
measurable HG-PRT activity, by producing an 
antibody to purified HG-PRT. It was found that only 2 
(or possibly 3) of the radiation-induced mutants lacking 
HG-PRT activity showed a cross-reaction to this 
antibody. However, it is again possible that these 
cross-reacting mutants are of spontaneous origin, since as 
many as 4 or 5 of the mutants selected after radiation 
treatment could be spontaneous mutants with no 
measurable HG-PRT activity (from the relative mutant 
frequencies and the fact that 50% of spontaneous 
mutants show no measurable HG-PRT activity, see 
above). None of these isolated mutants show loss of 
G6-PD activity, but a small number show changes in 
the X-chromosome arm known to carry the HG-PRT 
locus (see for instance [T31] for a description of a 
radiation-induced (P) mutation which has a clear 
X-chromosome rearrangement, probably a pericentric 
inversion of the Xq-X-chromosome with PDPs in 
the p2–q1 region).

604. Waldren et al. [W28] have presented some results 
from a study involving a CHO hybrid cell line 
containing a single human chromosome 11; on this 
human chromosome are genes for cell surface antigens 
a1, a2 and a3 and for lactate dehydrogenase-A (LDH-A). 
The a1, a2 and a3 loci cause the formation of specific cell 
surface antigens that render the cell sensitive to killing 
by specific antigens in the presence of the complement. 
The a1, a3 and LDH-A are on the short arm while a2 is 
on the long arm. In the mutagenesis experiments, the 
cells were either x-irradiated (100–600 R) or treated 
with MNNG and a1 mutants were isolated and tested, 
for loss of only a1, a1 and up to 2 others and all four 
makers. The results are summarized in Table 39 which 
shows that the frequency of single marker loss is quite 
low after x-rays (as compared with MNNG and 
controls): limited mutagenic effect for loss (a1 and up to 
two others) is higher for both x rays and MNNG (relative 
to controls). Total marker loss is highest in the x-ray 
group. These data therefore suggest that at least for the 
loci studied, the radiation-induced mutational events 
predominantly involve chromosomal deletions.

F. MUTAGEN-SENSITIVE CELL STRAINS

605. Recent years have witnessed an increasing 
interest in the isolation of cultured mammalian cell 
lines which are sensitive to specific mutagens. The 
imputus came from similar work with human cell 
strains derived from patients with inherited disorders 
and it is hoped that the isolation and study of mutagen-
sensitive mutants of cultured mammalian cell lines may 
also contribute to our knowledge of DNA repair 
processes and their relationship to mutagenesis. A 
number of mutagen-sensitive mutants have been 
identified in mammalian cell lines using replica plating methods [K41, S122], viral 
suicide method [S123] and others [T32].

606. Sato and Hieda [S82, S83, S84] have reported the 
isolation and characterization of mutant mouse cell 
strains isolated from LS178Y cells mutated with nitro-
soguanidine, which have been found to be sensitive to 
physical and chemical mutagens. Thus, for instance, 
the line designated as M10 [S83] which was originally 
isolated as being sensitive to methylmethane 
sulphonate, was later found to be more sensitive also 
to the killing effects of x rays. The UV sensitivities of the 
parental and mutant lines however, were similar, as 
were plating efficiencies and doubling times.

607. Shiomi et al. [S134] compared the gamma-ray 
induction of 6-TGR mutations in the LS178Y and the 
M10 lines and found that in the parental line the 
frequencies of induced mutations increased steadily 
with an increase in exposure (25–500 R; 2–3 10⁻⁷ R⁻¹); 
in the M10 line however, the frequencies increased in 
the range from 25–75 R, followed by a sharp decrease 
thereafter (100–150 R). In the lower exposure range, the
rate of induction per unit exposure in the M10 line was about 4 times that in the parental one. When the induced mutation frequencies were plotted against log survival, the relationship was linear only up to 20% survival in the M10 line (the curves for LS178Y and M10 were superimposable); in the LS178Y cells, a linear relationship was obtained over the whole range down to about 2% survival (see also [T23] and [T33]).

608. Another line isolated by these authors, designated as Q31 (also from LS178Y cells, after nitroso-
guanidine treatment) was sensitive to 4-nitro-quinolinone-
1-oxide (4NQQ), i.e., showed no growth in a plate
containing 50 ng/ml 4NQQ [S82]. Further tests revealed that
10 ng/ml of 4NQQ completely inhibited the growth of Q31 cells, but not that of parental cells. Q31 was also found to be more sensitive to the killing effects of UV (D0 of 1.6 J m⁻² versus 3 J m⁻²) but did not show any enhanced sensitivity to x-irradiation. Chromosome
analysis revealed that the cells of the parental line contained 41 chromosomes while those of Q31, 39 chromosomes.

609. In a subsequent study, Sato and Hieda [S84]
compared Q31 and LS178Y cells with respect to the induction of 6-TG⁺ mutations by UV and caffeine
sensitivity. The maximum yield of mutants was obtained after 7 days post-irradiation in LS178Y cells
and 14 days in Q31 cells. The mutation frequencies in
Q31 are higher after low doses of UV, but show a
decline after higher (more than 2 J m⁻²) doses (while
that of LS178Y continues to increase up to 12 J m⁻²).
A plot of the induced mutation frequency as a function of the
logarithm of surviving fraction again indicates hypermutability of Q31 cells as compared to the
parental strain. Caffeine affected the cell-killing effect of UV in both cell strains to a similar extent, indicating that the defective (repair?) process in Q31 was caffeine-
insensitive.

610. Thompson et al. [T32] have reported on the
isolation of mutagen-sensitive clones by mutagenizing
CHO cells with EMS, UV, MMC and ICR-171. Two of
the UV-sensitive clones studied in detail had a D₇遗留
of 1.0 J/m² compared to 7.0 J/m² for the wild-type
cells, and each was shown to have no detectable repair
replication following exposure to UV doses up to 26 J/m². Although these mutants resemble XP mutants in humans with respect to their repair defect and cross
sensitivity to the carcinogen 4-NQO, one of the two
clones is characterized by extreme hypersensitivity to
MMC (80-fold, as compared to the wild type). Clones
having hypersensitivity to alkylating agents but not UV,
were obtained using MMC and EMS. In the latter case,
the two clones had significantly increased sensitivity to
the killing effects of ⁶⁰Co gamma rays.

611. Busch et al. [B76] isolated 54 UV-sensitive clones of
CHO cells, including two from a parent cell line which
is hypersensitive to EMS and is also sensitive to x rays (EMS and ICR-170 were used to mutagenize
the cells). Most of the UV-sensitive clones studied thus far appear to be five-fold more sensitive than the original
strain, in terms of the slopes of the survival curves. All
the seven clones examined for DNA repair competence
using a repair replication assay (measurements of DNA repair using alkaline isopycnic gradients) exhibited a DNA repair defect (0-34% of normal) resembling that seen in XP cells. Furthermore, in two of the repair-replication defective clones, there was an approximately non-fold
enhancement of UV mutagenesis (Ouabain resistance,
6-TG⁺ and 8-azaadene).
619. Studies have been conducted to examine the effects of the combination treatment (PUVA) involving long-wave UV (UVA) and 8-methoxypsoralen (8-MOP). Treatment with 8-methoxypsoralen or UV (UVA) alone was not mutagenic and the combined treatments induced both mutations (6-TG<sup>R</sup>) and cell killing. The relationship between mutant yield and the product of the concentration of 8-methoxypsoralen times the UV (UVA) dose.

620. The autoradiographic method devised by Strauss and Albertini for quantitatively detecting 6-TG<sup>R</sup> variants in human peripheral blood lymphocytes is discussed together with the results obtained by these authors with lymphocytes sampled from patients with Lesch-Nyhan syndrome, normal individuals, and individuals who have undergone chemo- or radiotreatment. Evans and Vijayalaxmi have extended the usefulness of the lymphocyte system for detecting HG-PRT mutations in vitro studies using 8-azaguanine as the selection agent. X rays and mitomycin-C were the chosen mutagens. With both of these, increases in the yields of 8-AG<sup>R</sup> variants with increasing exposures were found. However, while the radiation-induced variants are considered to be mutational events, those induced by MMC are thought to represent cells in which large MMC-guanine adducts have prevented normal transcription of the HG-PRT locus to give functional HG-PRT.

621. In LS178Y mouse lymphoma cells, X rays and UV irradiation produce a linear increase in the frequency of thymidine kinase-deficient mutants with increasing dose. In the same system, unfiltered broadband spectrum radiation emitted by black light, cool white and black light blue, fluorescent lamps and a sunlamp are both toxic and mutagenic when the cells are irradiated in phosphate buffered saline. But their relative mutagenic efficiencies are different.

622. Gamma irradiation is ineffective in producing mutations to ouabain-resistance in Chinese hamster (V-79) cells, but UV irradiation or EMS treatment are effective in this regard. Lack of Ouu<sup>R</sup> mutation induction by ionizing radiation has also been demonstrated for CHO cells.

623. The effects of fractionated UV irradiation with cycloheximide or caffeine present during the interval between fractions on mutation induction to ouabain resistance have been studied. With cycloheximide present between the dose fractions the effects were different (an increase or a decrease in mutation frequency relative to the unfractiated UV dose) depending on the experimental protocol. Caffeine given as a pre-treatment or between the UV dose fractions always led to an enhancement of the mutation frequency.

624. There is now good evidence that a sizeable proportion of the HG-PRT mutations induced by ionizing radiations (in human and V-79 fibroblasts) is associated with structural changes of the X chromosome. With X rays, this proportion is of the order of 5–25%, whereas with high-LET radiations, it is of the order of 20–60%. Other studies using a CHO hybrid cell line containing a single human chromosome 11 (on which are present genes for cell-surface antigens, a<sub>1</sub>, a<sub>2</sub> and a<sub>3</sub> and that for lactic dehydrogenase-A) also provide evidence supporting the thesis that radiation-induced mutations at these loci may be predominantly chromosomal deletions.

625. A number of mutagen-sensitive cell lines have been isolated from cultured mammalian cells in recent years and these are proving to be useful in studies on DNA repair, relationship between DNA repair and mutagenesis, etc.

VII. EVALUATION OF GENETIC RADIATION HAZARDS IN MAN

A. A SUMMARY OF THE MAIN CONCLUSIONS REACHED BY THE COMMITTEE IN ITS 1977 REPORT

626. The 1977 report of the Committee presented the available human and experimental data that were considered suitable in the context of the evaluation of genetic radiation hazards in man; discussed the assumptions and uncertainties involved in making use of these data for such purpose; and gave quantitative estimates of hazards using both the so-called “direct” and the “doubling-dose” methods.

627. With the “direct method”, it was pointed out that, with respect to hazards from the induction of mutations, what was desired was an assessment of the risk of induction of mutations causing dominant effects in the progeny with some indication of handicaps and disabilities that they may cause. To do this, the mouse data on the gamma-ray induction of mutations causing dominant effects in the skeleton were used. The reasoning was, and still continues to be, that many of the skeletal abnormalities recovered in the mouse experiments are similar to rare dominants and rare irregularly-inherited dominant conditions in man which together constitute a sizeable proportion of human genetic diseases.

628. The overall estimate of risk arrived at using the data on skeletal mutations with appropriate corrections for low dose, low dose rate irradiation conditions, ease of diagnosis of skeletal defects in humans and severity of effects was 20 10<sup>-6</sup> per 10<sup>-2</sup> Gy (or 2000 10<sup>-6</sup> per Gy) of paternal, low-LET, low dose or low dose rate irradiation. In other words, 2000 individuals per million born will be expected to suffer from one or another serious handicap due to the induction of mutations with dominant effects per Gy of paternal radiation exposure under the stated conditions. The figure therefore subsumes the effects of mutations which are operationally classified as “dominants” as well as those of “recessives” which have effects in the heterozygous condition.

629. The rate of 20 10<sup>-6</sup> per 10<sup>-2</sup> Gy of paternal exposure was arrived at by the Committee by multiplying the estimated rate of induction of skeletal mutations 4 10<sup>-6</sup> per 10<sup>-2</sup> Gy (of low-LET, low dose rate irradiation) by a factor of 10 and then dividing the product by a factor of 2. The rationale was set forth as follows: "... Firstly, a perusal of McKusick's latest tabulation of monogenic disorders in man [M17] will reveal that out of the 583 'proved' autosomal dominants, 328 are clinically important: in the latter group, 74 (or roughly 20%) involve one or more parts of the skeleton to a varying extent. However, this figure is undoubtedly a reflection of the ease of diagnosis of such abnormalities by phenotypic inspection and/or radiography. The true figure therefore is likely to be lower and, in the opinion of Carter and McKusick, is of the order of 10%". (This means that only about 10% of
the dominant mutations in man are likely to be associated with skeletal defects. The reciprocal of this, i.e., 100/10, provides the multiplication factor 10 used to convert the rate of induction of skeletal mutations into one applicable to mutations having dominant effects on any of the bodily systems in man.)

630. "Secondly, not all of the skeletal abnormalities of mutational origin studied in the mouse will impose a serious handicap in humans. Selby suggests that this proportion of abnormalities (leading to serious handicaps) may range from 25% to 75%; in Carter's opinion, a figure of 50% may be accepted as a tentative estimate and this has been confirmed in a detailed discussion of the mutants by Selby and McKusick" [U1]. (This is the basis for dividing 10 x 4 10^-9 by 2.)

631. Similar data on the induction of skeletal mutations in mouse females were not available. The Committee carefully considered the existing evidence from specific locus studies in mouse females. In particular, the data showing that for radiation conditions applicable to man, maturing oocytes exhibit a very low level of mutational sensitivity and immature oocytes are virtually insensitive. It was argued that if the mutational sensitivity of human oocytes were similar to that of the mouse, the risk for human females will be low (and very much lower than that for males), but the Committee refrained from giving any quantitative estimate.

632. For computing the risk from the induction of balanced reciprocal translocations (the predominant kind of radiation-induced structural change), the Committee used the limited human and marmoset cytogenetic data obtained in experiments involving acute X-irradiation of spermatogonia. Two points deserve mention here: firstly, although, to be on the conservative side, the above data were used, it was noted that the sensitivity of the rhesus monkey (a species more closely related to man than either the marmoset or the mouse) to the radiation induction of reciprocal translocations in their spermatogonia is far lower than that of the marmoset or the mouse; secondly, it was assumed that balanced reciprocal translocations as such are not associated with adverse phenotypic effects of clinical significance and that the risks stem primarily from the unbalanced products generated during meiotic segregation in translocation heterozygotes.

633. Analysis of the cytogenetic data (obtained in experiments involving irradiation of the testes of marmosets and men and screening of the spermatocytes descended from irradiated spermatogonia) permitted an estimate of about 7.10^-4/10^-2 Gy/cell. From this, the rate of heritable translocations was estimated as 1.75 x 10^-4/10^-2 Gy/gamete (assuming that the rate of recovery in the F1 will be one-fourth of that in spermatocytes). The rates for low dose X rays, low dose rate X rays and chronic gamma-irradiation were derived by dividing the above figure of 1.75 x 10^-4 by 4, 2 and 10, respectively. The rates thus derived (0.44 x 10^-4, 0.88 x 10^-4 and 0.18 x 10^-4/10^-2 Gy/gamete) were used to estimate the proportion of unbalanced zygotes that will result (= twice the above rates; based on the segregational properties of translocation heterozygotes) and the proportion of the unbalanced zygotes that will result in children with multiple congenital anomalies (= 6% of unbalanced zygotes; based on human data: see [U8]). These calculations showed that per 10^-2 Gy of paternal irradiation, between 2 and 10 per million children born will suffer from multiple congenital anomalies which can be attributed to the induction of reciprocal translocations in the spermatogonia of irradiated males.

634. The Committee stressed the point that the rates of translocation induction used above were based on human and marmoset cytogenetic data with corrections for transmission and expected effects at low doses and dose rates being taken on mouse data. It cautioned that "...should it turn out that the rate of induction in human spermatogonia is more similar to that in the rhesus monkey, the estimates may need revision, and consequently the quantitative figures arrived at must be considered provisional at present".

635. There were no data on the rate of translocation induction in human females or in any primate species. The data for mouse females showed that in maturing oocytes exposed to acute X-irradiation, the rate was 0.16 x 10^-4/10^-2 Gy/gamete (semi-sterility data). The Committee stated that "...although there is no direct evidence on the response of immature oocyte stages to the induction of translocations at low doses and dose rates, the data on specific locus mutations and on X-chromosome losses strongly support the view that the rate for translocations is also likely to be low, but no quantitative estimates can be given".

636. The Committee expressed the view that the risk from the induction of structural aberrations other than reciprocal translocations is likely to be very small since most of them act too early to constitute a real hazard. For chromosomal deficiencies (which are also known to be induced at relatively high frequencies in the mouse after oocyte irradiation) it was stated that "...not enough is known yet about their probable over-all rate of induction or deleterious effects (especially in the heterozygote) to make any estimate of the genetic damage they may cause".

637. The risk from the induction of sex-chromosome losses was considered to be very low after irradiation of males and probably also very low after irradiation of females, these conclusions being based on mouse data. The Committee carefully evaluated the human as well as mouse data on the induction of non-disjunctional effects and concluded that information on this is very inadequate or conflicting and therefore no quantitative estimates could be given.

638. For arriving at estimates of risk using the "doubling-dose method" the Committee made use of the following assumptions and data. The doubling-dose for all genetic effects is probably of the order of 1 Gy and the use of this figure in computations is not likely to underestimate risk. The incidence of autosomal dominant and X-linked diseases in man is 1%; the increase in the frequency of these diseases as a result of radiation exposure will be directly proportional to the mutational rate. Autosomal recessive diseases occur in humans at a frequency of 0.1%; their incidence is only very indirectly related to mutation rate. The incidence of numerical chromosomal anomalies and unbalanced structural anomalies is of the order of 0.4%; the increase in their frequency will be directly proportional to mutation rate. Balanced reciprocal translocations per se may not confer any risk to the carriers; the risk from the induction of such structural rearrangements mainly stems from the unbalanced products generated by these during meiotic segregation. For diseases of complex aetiology, such as irregularly-inherited dominant diseases, multifactorial diseases, and congenital malfor-
mations (together constituting 9%), the mutational component (i.e., the proportion of these diseases that will respond to radiation exposure in a manner similar to that of simple monogenic dominant diseases) is of the order of 5%. Lastly, under continuous radiation exposure \((10^{-2} \text{ Gy/generation})\), the population will reach a new equilibrium with respect to the incidence of these diseases: the rate of approach to the equilibrium as well as the incidence values at equilibrium and in the first generation following radiation exposure will be different for the different classes of diseases; thus for instance, the approach to equilibrium will be faster for single-gene dominant diseases, very slow for recessive diseases and somewhat intermediate for diseases of complex aetiology. The first generation incidence following radiation exposure (under conditions of continuous radiation exposure) will be one-fifth of that at equilibrium for single gene dominant traits and X-linked diseases, nearly the same in the first generation and at equilibrium for chromosomal diseases and one-tenth of that at equilibrium for diseases of complex aetiology.

639. All these considerations permitted an estimate of the total increment expected in the first generation amounting to 63 new cases of genetic diseases per million progeny and 185 cases per million at equilibrium, when the population is continuously exposed to low dose rate, low dose or chronic low-LET irradiation at a rate of \(10^{-2} \text{ Gy per generation}\).

B. RELEVANT NEW INFORMATION THAT HAS BECOME AVAILABLE SINCE THE PUBLICATION OF THE 1977 REPORT

640. The new data that have become available since the publication of the 1977 report (and considered in detail in the preceding chapters) can be arbitrarily grouped under at least four categories, with some overlap between them: (i) those that confirm and further document the Committee's earlier conclusions; (ii) those that help to shed light on the validity of assumptions or tentative conclusions (arrived at on the basis of limited data) or controversial view-points; (iii) those that seem relevant in a qualitative sense but which, as yet, cannot be used in a quantitative manner for risk evaluations; and (iv) those which open up potentially useful approaches to hazard evaluations and/or those which may be of use in improving quantitative evaluation of hazards. Some of the most important of these will be summarized in the following paragraphs.

1. Confirmatory data

(a) Human studies

641. The incidence figures for congenital malformations in the Hungarian survey are similar to the estimate made by Trimble and Doughty in the British Columbia survey. The data of Myrianthopoulos and Chung based on an almost complete ascertainment of cases of congenital malformations show that, if anything, their incidence may be even higher. The analysis of the transmissibility of some common congenital malformations summarized by Leck (for all these, see section I.A) serves to illustrate the view expressed by the Committee, namely, that the mutational component of these diseases is probably quite small.

642. The new data from the recent Edinburgh and Japanese surveys on the incidence of chromosomal abnormalities in newborns (subsection I.C.1. Table 2) show that, despite the systematic use of banding techniques, the frequencies of different kinds of abnormalities are similar to those reported in a number of earlier surveys. The currently available results from chromosomal studies of children born to survivors of Hiroshima and Nagasaki (subsection I.C.11) show that the frequencies of chromosomal abnormalities are similar to those in controls.

643. Reviews on the incidence of chromosomal abnormalities in spontaneous abortions and aneuploidy (section I.C, Figure 1) document and confirm that:

(a) The overall frequencies of chromosomal abnormalities in spontaneous abortions may be as high as 50%.

(b) Spontaneous non-disjunction plays a major role in generating aneuploidy.

(c) Such aneuploidies are frequent among chromosomally abnormal foetuses (sub-section I.C.4) and in newborns (Table 2).

(d) Non-disjunction occurs in the male as well as female germ cells, both in meiosis I and in meiosis II (subsection I.C.7 and Table 4), as suggested by studies on spontaneous abortions and on Down's syndrome.

(e) Evidence for radiation-induced non-disjunction in man continues to be equivocal (Table 8).

(f) Direct studies on aneuploidy in human spermatocytes now appear possible (subsection I.C.10).

644. Many known single-gene traits in humans are associated with neoplasia (section I.D., Table 9). DNA repair studies with human cells (subsection I.E.3) which have been in progress for some years now, have underlined the important role of these processes, particularly with respect to their relationships to cellular response to radiation and other mutagenic agents (subsection I.E.2).

(b) Studies with experimental organisms

645. Further evidence has been obtained in mice for the induction of genetic effects by \(^{239}\text{Pu}\) (section II.A). Even low doses from \(^{239}\text{Pu}\) alpha rays are capable of causing significant increases in the frequency of translocations (subsection II.B.2). Studies with \(^9\text{H}\) (given as tritiated water) have shown significant induction of specific locus mutations in both pre- and post-mitotic germ cells of male mice (subsection II.D.1); the mutational spectrum shows no striking disparity from that induced by x rays.

646. The dose-response curve for the induction of specific locus mutations in maturing oocytes of female mice (high dose rate x-irradiation) has now been extended to include the 6 Gy dose point. All the high dose rate data obtained at different doses to maturing oocytes considered together confirm that the dose-response is curvilinear (subsection II.D.2).

647. Confirmatory evidence for earlier findings that the yield of reciprocal translocations induced in spermatogonia decreases with decreasing dose rate of low-LET radiation has been obtained in a new set of studies (subsection II.B.2; Table 21). Further data on the induction of heritable translocations in female mice are in line with earlier results in showing that the rate in maturing oocytes of females is about one-half of that in
males (subsection II.B.3). The frequency of chromatid interchanges induced in maturing mouse oocytes (scored in metaphase I) is lower by a factor of 10 at low dose rate relative to that at high dose rate (subsection II.B.4).

648. The weight of currently available evidence suggests that, following irradiation of male mice (spermatogonial irradiation), Robertsonian translocations are recovered (if at all) at very low frequencies.

649. A number of studies have further documented the existence of differences in sensitivity for the induction of chromosomal aberrations in male and female mouse germ cell stages (subsections II.B.2 and II.B.4) and of dominant lethals (subsection II.A.1 and Tables 20 and 22). The new data on dominant lethal induction in female guinea pigs. Djungarian and Chinese hamsters (subsection II.A.2) serve to further highlight the existence of species differences in radiosensitivity.

650. A number of studies have been carried out on the induction of aneuploidy by irradiation of male and female mice and female Chinese hamsters (subsection II.C.4). While in mice in some studies the evidence for the radiation-induced aneuploidy (scored in utero) appears good, in others it is weak; in Chinese hamsters, the evidence is not clear-cut.

651. Completed studies on x-ray-induction of reciprocal translocations in the spermatogonia of rhesus monkeys show that the dose-response is “humped” with a peak around 1 Gy (thus being qualitatively similar to what is known for other mammals studied in this respect) and with a rate of induction (up to doses of 1 Gy) being substantially lower than what has been recorded for other mammalian species (subsection II.B.5; Tables 23 and 24).

652. Studies on the acute x-ray induction of autosomal recessive lethals in maturing oocytes of mice have demonstrated that the rate is nearly the same as that for spermatogonia. Germ cell stages in female foetuses do not appear to be more sensitive to the x-ray induction of autosomal recessive lethals than maturing oocytes in adult females. There is a dose rate effect for the induction of specific locus mutations in oocytes of female mice irradiated shortly before birth, suggesting that the repair capacity of these oocyte stages are not different from that of maturing oocytes in adult females (section II.D).

653. Further data on the relationship between chromosome arm numbers and relative radiosensitivity to the induction of dicentrics in lymphocytes confirm the view expressed in the 1977 report, namely, that chromosome arm number is not a reliable criterion for extrapolation from one species to another with respect to the sensitivity of the chromosomes to the induction of dicentrics (subsection II.F.2).

654. Irradiation of mice or rats at very low dose rates (of the order of 1 \(10^{-2}\) to 2 \(10^{-2}\) Gy d\(^{-1}\), gamma rays) causes no reduction in spermatogonial stem cell numbers, although at rates \(> 3 \times 10^{-2}\) Gy d\(^{-1}\), the situation is different (chapter IIII). Further data from labelling studies of irradiated mouse testis show that the surviving spermatogonial stem cell population is qualitatively the same for that portion of the dose-response curve giving a linear increase in mutation frequency (up to 600 R acute irradiation).

655. Studies on the timing of oocyte maturation in the mouse show that a period of six weeks elapses between the initiation of stage 3b to ovulation. This means that in mutation experiments, conceptions within six weeks after irradiation would utilize oocytes exposed in stages 3b-8 and that litters conceived after six weeks would be derived from stages 1-3a. Since the nuclear morphology of both stages 3a and 3b is similar to one another and also to stage 3 of guinea pig and human oocytes, it would appear that the change in mutational sensitivity of the mouse oocytes occur in a cell stage with “typical” diplotene configuration (chapter IV).

656. Evidence has been obtained that in human fibroblasts and Chinese hamster cells, a significant proportion of x-ray and high-LET radiation-induced H2-PRT mutations is associated with cytologically demonstrable deletions or other aberrations in the region of the H2-PRT locus (section IV.A).

2. Data that shed light on the validity of assumptions, tentative conclusions and controversial viewpoints

657. The interpretation of the dose-rate effect observed for the induction of specific locus mutations (and thus, of the nature of the mutations induced) in experiments with female mice has been controversial for some time. While W.L. Russell has continued to favour the hypothesis that the mutations themselves are single-track phenomena and other track events are involved in damaging or saturating the repair process at high doses and dose rates, others (Abrahamson et al. Brewen et al.) have preferred an interpretation based on the premise that the mutations are predominantly two-track phenomena. In the discussion of their recent experimental results on dose-effect relationships for specific locus mutations in maturing mouse oocytes, Lyon et al. have supported W.L. Russell’s interpretation and have detailed their reasoning for this (subsection II.D.3).

658. The question of whether the genetic radiosensitivity of the immature oocytes of the human female is similar to that of the mouse has been kept under continual review by the Committee (see, for instance, paragraphs 293–298 in the 1977 report). On the basis of all the evidence available the Committee concluded in 1977 that there was no consistent correlation between the induction of genetic effects, cell killing and plating and some morphology in oocytes (in mice, guinea pigs and golden hamsters); that even if one continues to consider the possibility that the human immature arrested oocyte might be mutagenically as sensitive as the most sensitive of all oocyte stages in the mouse, for radiation conditions applicable to man the genetic hazard of radiation in the female will still be less than in the male. The new data from studies on the timing of oocyte maturation in the mouse (chapter IV) support the earlier view that nuclear morphology is not a reliable criterion of oocyte sensitivity: the nuclear morphologies of stages 3a and 3b oocytes are similar to one another (and also similar to those of stage 3 human and guinea pig oocytes) and yet, strikingly different in mutational sensitivity.

659. The results of experiments in Drosophila and Escherichia coli have yielded results consistent with one of the main assumptions involved in the use of the “doubling dose” method for risk evaluation, namely, that there is proportionality between spontaneous and induced rates of mutations (subsection II.F.4). However, the consistency in Drosophila exists, so far,
only for induced mutations in spermatozoa, and the consistency in Escherichia coli is dependent on excluding "hot-spots".

660. It may be recalled that in making estimates of increase of dominant and X-linked diseases (using the doubling dose method) under conditions of continuous low-LET radiation exposure at the rate of 10⁻³ Gy/ generation, the Committee in its 1977 report assumed that the first generation increment for these diseases will be about one-fifth that at equilibrium. The recent calculations of Childs [C68] show that the increment in the first generation is of the order of 15% of that equilibrium, a figure which is quite similar to the 20% used by the Committee.

3. Data that seem relevant in a qualitative sense

661. The frequencies of chromosome aberrations in lymphocytes of individuals living in an area of high natural radioactivity in Austria have been found to be elevated; this is also true of nuclear dockyard workers in the United Kingdom, of nuclear power plant workers in the Federal Republic of Germany and the United Kingdom, of uranium miners, of workers with internal depositions of plutonium, and of atomic bomb survivors of Hiroshima (section I.F).

662. Studies have been made with rabbits and mice keeping them artificially in huts in areas of high natural radioactivity in France for periods up to 20 months (only four months in the case of mice). In rabbits there was an increase in the frequencies of chromosomal aberrations (lymphocytes) reaching up to about 4% by one year; however, by 20 months, there were practically no aberrations. Male mice kept for four months, showed no translocations in their spermatocytes. But, when such "irradiated" males were mated to unirradiated animals, there were slight increases in litter size, the mean number of offspring sired and the mean number of offspring weaned over a six-month period. Female "irradiated" mice in contrast, under similar conditions showed slight reductions in all of the above indicators (subsection I.F.1).

663. Although it has been known for a long time that certain classical chromosomal syndromes in man are associated with congenital malformations, mental and physical defects, it is only in recent years that studies are beginning to focus attention on the possible clinical significance of other abnormalities such as balanced reciprocal translocations. The currently available data, although not based on random samples, are suggestive of an association between apparently balanced reciprocal translocations (and some other structural rearrangements) and mental and physical defects (subsection I.C.2) and point to the need for further studies (e.g., follow-up of children studied as newborns and who were found to have balanced structural anomalies).

664. In the mouse it has also been shown that some balanced reciprocal translocations can have dominant phenotypic effects on the skeleton (subsection I.D.8).

665. The preliminary results from tests of mutants originally isolated because of the skeletal abnormalities that they caused in the F₁ offspring, show that most of them behave as recessive lethals. Put another way, radiation-induced recessive lethals can have dominant deleterious effects (subsection I.D.8).

666. The utilization of banding techniques to study human chromosomes has, among other things, led to the discovery that partial monosomies and trisomies for practically every chromosome of the human complement are frequent, and are in fact more common than was envisaged a few years ago (subsection I.C.12). The recent discoveries of heritable fragile sites in human chromosomes have exposed another facet of chromosome structural defects (?) whose significance in the context of human pathology remains to be established.

667. Studies of chromosomal evolution in primates using banding techniques have shown that the sequence and types of chromosomal changes in different lines are quite different (subsection I.F.5). However, the questions of whether the predominance of a particular kind of change in the evolutionary history of a given species (for instance, the predominance of pericentric inversions in the line of descent leading to Homo sapiens) puts some constraints on or predisposes the species to similar inducible changes or whether the evolutionary history is "irrelevant" in the context of studies on induced aberration remain to be answered.

668. The arguments advanced by Neel in favour of the thesis that the contribution to human disease of mutations technologically classified as recessives is currently underestimated, have been considered (section I.A); however, at present, it is difficult to give quantitative estimates.

4. Data useful for quantitative assessment of genetic radiation hazards

669. The only new data that have become available since the publication of the 1977 report and which can be used in the context of quantitative assessment of genetic risks to man are those that derive from experimental studies on the induction of dominant mutations causing cataracts in mice (subsection I.D.8). Further, the data on the induction of reciprocal translocations in the rhesus monkey (subsection I.B.5), although not new, can be used, at least to set an arbitrary lower limit for the risk from the induction of reciprocal translocations.

C. SOME RELEVANT RECENT PUBLICATIONS ON QUANTITATIVE EVALUATION OF GENETIC RADIATION HAZARDS IN MAN

670. In two papers, Selby [S75, S117] has discussed, among other things, the problem of quantitative evaluation of genetic radiation hazards in man and has briefly summarized the risk estimates (including the methods and assumptions used) arrived at by UNSCEAR in its 1977 report and by the BEIR Committee in its report published in 1980 [B77]. The estimates of the latter Committee are discussed later in this section and will not be gone into here.

671. Ehling [E17], Ehling and Kratochvilova [E13] Kratochvilova and Ehling [K23] made a preliminary estimate of genetic risks from the induction of mutations having dominant effects, using the data on cataract mutations in mice obtained in their experiments in which a gamma-ray exposure of 910 R was given to male mice in two equal fractions separated by a 24 h interval (sporomagogonial data). As will be recalled (subsection I.D.8), the rate of induction
estimated in this study was $1.3 \times 10^{-6}$/gamete/R. This figure was multiplied by 0.85 (to correct for the enhancement effect due to fractionation and based on specific-locus data collected in the same study), by 0.3 (correction factor for dose rate effect), by 1.2 (to correct for DNA content in human cells relative to that in mouse cells) and by 32.4 (to extrapolate from data on dominant cataract mutations to total dominant mutational damage affecting all bodily systems; based on the ratio of dominant cataract mutations to all dominant mutations as estimated from the tabulations of McKusick in 1975 [M17]). The resultant figure was $13 \times 10^{-6}$. In other words, following paternal low-LET irradiation, there will be 13 progeny per million born (per hit of irradiation) who would be expected to be affected with one or another kind of serious genetic disease of induced mutational origin.

672. In subsequent papers, Ehling [E20] and Ehling et al. [E24] extended these calculations to data from studies on the induction of cataract mutations by single acute gamma-ray exposures of 534 R and 600 R. As discussed earlier (Chapter 11.D.8), the rates of induction estimated from these data are: 5.5 $10^{-7}$/gamete/R (534 R) and 4.3 $10^{-7}$/gamete/R (600 R). These rates were multiplied by 36.8 (the "new" ratio of cataract to all dominant mutations that can be calculated from McKusick's more recent compilation of 1978 [M16]). The resultant figures were: 20 $10^{-6}$/gamete/R and 17 $10^{-6}$/gamete/R. In other words, following paternal, low-LET, high dose rate irradiation, 17–20 affected progeny per million per R of exposure will be expected in the F1. The figures will be one-third of these for low dose rate, low-LET irradiation.

673. In a recent paper, Childs [C68] has made a detailed analysis of published data on birth frequencies, mutation rates and fitness of each of the important dominant and X-linked diseases in humans. For dominant diseases, the author made use of the list compiled by Carter [C49, C50] with minor modifications. The frequency of rare dominant diseases was estimated based on the frequency of diseases given by Trimble and Doughty [T1]. For X-linked diseases, the source was the list compiled by Stevenson and Kerr [S124] and selected data from Trimble and Doughty.

674. From his analysis, Childs estimated that these diseases (dominant and X-linked) affect about 0.6% of liveborn individuals of which 16% or 0.1% of livebirths carry a newly-arisen mutation. Assuming that the population is exposed to low-LET irradiation at a rate of $10^{-2}$ Gy/generation and that the doubling dose is 1 Gy, he estimated that at equilibrium, there would be about 61 extra cases per million livebirths of serious genetic disease; the expected increase in the first generation following the radiation exposure would be 15% or 9 extra per million. The increase in the first two generations would be 24% of that at equilibrium and a 50% increase would occur by the ninth generation. For dominant diseases alone, the increase would be 14% in the first, 23% by the second and 50% by the tenth. For X-linked diseases alone, the increase in the first generation is 25%, and in the first two generations, 50%. (As X-linked diseases have a birth frequency of only 4% of that of dominant diseases, the combined increase is only slightly higher than for dominant diseases alone.)

675. Childs considers that the estimates arrived at above may be maximum estimates for two reasons. Firstly, Neel et al. [N24] calculated that the minimum doubling dose, based on mortality data of the children of atomic bomb survivors of Hiroshima and Nagasaki, and mutation induction in mice at low dose rates, should be 1.38 Gy for males and more than 10 Gy for females, i.e., a harmonic mean of about 2.4 Gy. Secondly, some of the dominant disorders included in the analysis (the two common disorders Marfanoidism and prolactinemia and otolesion and otsclerosis account for 50% of the birth frequency of dominant disorders) are likely to be maintained by selection pressure alone and would therefore not change in frequency with a change in mutation rate [N25]. Childs concludes that the "overall dominant genetic risks from ionizing radiation may have been overestimated by UNSCEAR (1977) by a factor of at least 6". Thus, using a doubling dose of 2 Gy and excluding the disorders mentioned above and X-linked ones, the expected extra number of defective children would be 3 per million births and 12 per million births at equilibrium.

676. Other papers of interest are those of Neel, Schull and Otake [N21], Schull, Otake and Neel [S130] and Schull et al. [S131], the last of which is the most recent. In these papers, the authors presented an analysis of all the currently available genetic data obtained from continuing studies of the Hiroshima and Nagasaki populations. The data pertain to untoward pregnancy outcomes, survival through childhood, incidence of sex-chromosomal aneuploids and incidence of biochemical variants. In all the calculations, an RBE of 5 for neutrons has been assumed. As Schull, Otake and Neel [S130] point out "in no instance is there a statistically significant effect of parental exposure. But for all indicators, the observed effect is in the direction suggested by the hypothesis that genetic damage resulted from the exposure". Doubling dose estimates have been made only for the first three of the indicator traits since the data on biochemical variants are considered too preliminary to provide a meaningful estimate for this end-point.

677. The gametic doubling dose estimates presented [S131] are the following: untoward pregnancy outcomes: 0.69 Sv (standard deviation, 0.93); survival through childhood: 1.35 Sv (standard deviation, 3.88); sex-chromosomal aneuploids: 5.35 Sv (standard deviation, 24.31). The weighted average of the estimates is 0.09 Sv with a standard deviation of 1.56. The authors [S130] consider that this estimate should be multiplied by a factor of 3 to convert it into a relevant estimate for low doses and dose rates.

678. The Committee examined the data and the analysis presented and concluded that in view of the lack of statistically significant effects and high standard deviations associated with the doubling dose estimates, it would be premature to use these for genetic risk assessments at the present time.

679. Genetic risk assessments for radiological protection purposes. Ofstedal and Searle [O18] have summarized the conclusions of a Task Group set up by ICRP (hereafter to be referred to as Task Group) to make a quantitative risk assessment and to express the risks in terms comparable to those for somatic risks. Most of the main conclusions reached by the Task Group are similar to those of UNSCEAR in its 1977 report. This is to be
expected in view of the fact that the basic data and several of the assumptions used are similar in both cases. To facilitate easy reference the summary table of the Task Group is reproduced below as Table 40.

680. UNSCEAR used both the direct and the doubling dose method for the computation of risks. The Task Group, however, used the doubling dose method (except for reciprocal translocations to be discussed later) because "(i) it allows the risk to be expressed in terms of basic human hereditary damage and (ii) if an overall genetic doubling dose is accepted, it allows an overall genetic risk assessment to be made even in the absence of direct experimental evidence on certain categories of hereditary defect'.

681. In the UNSCEAR 1977 report, the risks (calculated using the doubling dose method) are expressed as a certain number of cases of serious genetic disease per million progeny following low-LET, low dose or dose rate irradiation of a population (assumed to be stable in size) at a rate of $10^{-2}$ Gy/generation. The Task Group has expressed the risks, instead, as a certain number of cases per million man-rem (which is another way of expressing them, allowing for the possibility of exposure to different kinds of irradiations with different RBEs and LETs).

682. The doubling dose used by UNSCEAR is 1 Gy (low-LET irradiation) and that by the Task Group 100 rem; with this method, the Committee computed the expected increments for the different categories of genetic disease as a certain number of cases in the first generation and at equilibrium. The Task Group gave in addition, figures for the second generation as well.

683. For simple dominants and X-linked diseases, the expected increments in the first generation and at equilibrium computed by the Committee and by the Task Group are the same (namely 20 per $10^6$ in the first generation and 100 per $10^6$ at equilibrium).

684. For diseases of complex aetiology (incidence figure of 9% or 90 000 per 109), the Committee assumed that the mutational component is of the order of 5%, which is another way of saying that the incidence of only a small fraction of these diseases (5% of 90 000 per $10^6 = 4500$ per $10^6$) will respond in direct proportion to mutation pressure (just like the simple dominants and X-linked ones) while with the bulk of these, radiation would not produce any increase in their incidence rates. Thus with a doubling dose of 1 Gy, and under radiation exposure at the rate of $10^{-2}$ Gy/generation, the expected increase at equilibrium will be 45 cases per $10^6$ individuals born (i.e., $4500 \times 1/100 = 45$). The Committee made the further assumption (based on the BEIR Report [860]) that one-tenth of these cases (or about 5) will be expressed in the first generation.

685. The Task Group split up the category of diseases of complex aetiology into dominants of incomplete penetrance and multifactorial diseases maintained by mutation and multifactorial disease not maintained by mutation and stated that "these two categories of genetic damage are very difficult to study and their birth frequency in the population cannot be determined with any degree of assurance." That is, they did not use the incidence figure of 9% (for both categories together) arrived at in the British Columbia Survey. Consequently, the expected increments after radiation exposure were arrived at in a way different from the one used by the Committee described above. The Task Group "... felt that the number of extra cases at equilibrium after a parental dose of 1 million man rem could be taken as the sum of the equilibrium values of the categories (unbalanced translocations, aneuploid conditions and simple dominants), that is 100 + 30 + 30 = 160 of which 10% would become manifest in the first generation after a single exposure..." (see Table 40). For the second category described above the expected increment was given as zero. Thus, one of the major discrepancies between the conclusions of the Committee (1977 report) and of the Task Group (45 cases per $10^6$ versus 160 cases per $10^6$) stems from the different assumption and the way of calculating the effects of irradiation on the category of diseases with complex aetiology.

686. Regarding diseases with a recessive form of inheritance, the Committee in its 1977 report expressed the opinion that the effect of low level irradiation on the incidence of these diseases in the first generation will be "relatively slight" while at equilibrium (which will be attained after hundreds of generations) there will be a slight increase.

687. The Task Group used the same lines of reasoning as those used by the Committee in its 1977 report as will be clear from the following statements: "... The probability of a newly induced recessive mutation pairing up with a previously existing mutant allele at the same locus, so that the deleterious condition is expressed in the first generation after exposure, is regarded as negligibly low. In general, homozygous effects of induced recessives will be spread over hundreds of generations, with a very slow attainment of equilibrium ... for the above reasons, and in the context of radiation protection, it was thought that the true recessives would probably not contribute significantly to the extra mutation load following low level radiation exposure...".

688. The Task Group however mentioned that "... the evaluation of recessive mutational effects is very difficult and, not surprisingly, members of the Task Group were not unanimous in their views on this subject ... Two corresponding members have stated that the risk from 'nominal' recessive disorders, although largely concealed for many generations, may be a far graver problem than all other hazards combined, especially if these so-called recessives have significant effects in the heterozygotes. However, in the present state of our knowledge, this aspect of the risk problem is extremely difficult to quantify ...".

689. Turning now to chromosomal diseases, the Committee in its 1977 report used an incidence figure of 0.4% (sex-chromosomal and autosomal trisomies, XO, mosaics and unbalanced structural aberrations) and estimated that with a doubling dose of 1 Gy and radiation at the rate of $10^{-2}$ Gy/generation, there will be about 40 cases of affected individuals per million; since the reproductive fitness of these individuals will be zero, there will be no accumulation over generations and thus the effects seen in any given generation will be essentially those of new chromosomal changes of the above kinds.

690. For the Task Group, the starting point, i.e., the incidence figure for these diseases was 0.5% (the overall frequency of sex-chromosomal and autosomal trisomies and the XO); this figure was multiplied by a factor of 0.6 to take into account the possibility that the frequency of transmission (to the gamete) of aneuploid
conditions may be lower after treatment of spermato-
gonia than of oocytes, as has been found in experi-
mental studies with mice. It was stated that "... since
all these aneuploids will be manifest in the first
generation and will not reproduce, the expected
frequency of extra cases for a doubling dose of 100 rem
will be 0.005 x 0.6 x 0.01 per rem, which is a risk of 30
per million in the offspring of a person receiving 1
rem".

691. In the 1977 report, the risk of producing unbal-
anced gametes leading to congenitally malformed
children (stemming from the induction of balanced
reciprocal translocations in males) was estimated using
the combined marmoset and human cytogenetic data
as 2 to 10 per million per rad. The risk for the irradiation
of females was considered to be low, but no quanti-
tative estimates were given.

692. The Task Group however, used the same data
base (marmoset and human data) and gave an estimate
for only low dose rate x-irradiation, i.e., a reduction
factor of 0.5 was used noting "that the human x ray
doses were below 100 rad". The rate so obtained was
doubled to take into account irradiation of males and
females. The rationale for this procedure was set forth
as follows: "Heritable translocations can be induced in
female and male germ cells by x-irradiation of dicentric oocytes.
Although mutation rates are similar to those after
spermatogonial irradiation, it seems probable from the
results of specific locus experiments that the effect of
lowering the dose rate will be more pronounced in
oocytes than in spermatogonia. On the other hand,
because translocations induced in oocytes would be in
the form of chromatid exchange, a gamete from an
affected oocyte would be six times more likely to have
an unbalanced form than a balanced one [S135]. It
seems reasonable to regard these opposing factors as
cancelling each other out. Therefore, the risks from
translocation induction in female germ cells have been
taken to be similar to those in male ones." It will thus
be clear that the nearly two-fold higher risk estimated
by the Task Group derives from the assumption of
equal sensitivity of males and females, one which was
not used by the Committee.

693. The BEIR Committee's assessments. To facilitate
discussion, the summary table from the above
Committee's 1980 report [B77] is given below (Table
41).

694. Two methods have been used. The first is the
indirect relative-mutation-risk method used by the
BEIR Committee in its 1972 report (and what in
UNSCEAR reports is referred to as the doubling dose
method) and the second, the direct method for
estimating total phenotypic damage induced in a single
generation. With the first method, the estimates are
expressed in terms of risk per rem of added exposure.

695. The BEIR Committee adopted a range for the
relative mutation risk of 0.002 to 0.004 per rem (doubling
dose, 50-250 rem). As is pointed out "this is based
mainly on our best substantiated estimate of the
doubling dose, namely, 114 R for mouse spermatogonia
(for x- and gamma-radiation, the Roentgen, R, and
the rem are virtually equal). We approximately halve
and double this to get our range of 50-250 R, which we
believe overestimates the true value. Further reason for
thinking that this range is broad enough comes from the
estimates of 100-200 R obtained when data from both
sexes are combined ... The few human data suggest
that humans are not notably more sensitive, and are
probably less sensitive than mice".

696. For autosomal dominant and X-linked traits, the
range of 40-200 (Table 41) under the heading
"equilibrium" has been arrived at by multiplying the
current incidence figure of 10,000 (per million) by the
relative mutation risk range of 0.02-0.004 mentioned in
the preceding paragraph. Although no corresponding
figure for the first generation is given (see Table 41), it
is pointed out that "... if we were to use the BEIR 1
method of estimating first generation expression from
equilibrium estimates, then a mean persistence of 5
generations would imply first-generation expression in
the range of ... 8-40 per million liveborn per rem of
parental exposure".

697. For irregularly inherited diseases, the BEIR
Committee assumed that the mutational component of
these diseases is in the range of 5-50% (the same as that
used in their 1972 report). When the current incidence
(9%) is multiplied by this range as well as by the relative
risk range, the range of 20-900 given in Table 41 is
obtained. Again, the BEIR Committee did not give the
increment in the first generation. However, it was stated
that "... these mutants would be expected to persist for
'equilibrium' periods than would the simple autosomal
dominants. BEIR 1 assumed a mean persistence of 10
generations, which would lead to an expectation of a
first generation expression of about one-tenth the
equilibrium expression".

698. For autosomal recessives, the conclusions of the
BEIR Committee (see Table 41) are essentially the same
as they reached in their 1972 report (this was also
discussed in the 1977 report of UNSCEAR).

699. Turning now to the range of 5-65 cases per
million per rem given in Table 41, this represents the
direct estimate of the total phenotypic damage in the
first generation. The skeletal data obtained by Selby
and Selby and by Ehling in mouse studies were used for
this purpose and this was also the case with the estimate
of UNSCEAR. The estimate of UNSCEAR was
however 20 cases per million per 10^-2 Gy of paternal
irradiation. The reasons for the difference in the quanti-
tative estimates are the following.

700. First, to convert the rate of induction of skeletal
mutations in mice to an overall rate involving all bodily
systems in humans, the UNSCEAR used a multipli-
cation factor of 10 (i.e., 4 10^-4 x 10 = 40 10^-6). This
was divided by a factor of 2 to exclude mutations whose
effects are slight; the BEIR Committee on the other
hand, used a range of 5-15 to make the first conversion
and used a range of 0.25 to 0.75 to make the second
conversion. In its view, these ranges reflect the uncer-
tainties involved. These operations (i.e., 4 10^-4 x 5 x
0.25 and 4 10^-4 x 15 x 0.75) give a range of 5-45. In
other words, following paternal low-LET, low dose rate
irradiation at a rate of 1 rem per generation, the
expected number of individuals (in the generation
following the exposure) who will suffer from the effects
of serious genetic diseases of induced mutational origin
will be in the range of 5-45 per million livebirths.

701. Second, to take into account the effects of irradi-
ation of females, the BEIR Committee multiplied the
upper limit of 45 given above by 1.44 (the UNSCEAR
1977 report assumed that the risk for irradiated human
females will be negligible and did not give any quanti-
tative estimate). The rationale for this multiplication
was set forth as follows: "The mutational response of resting oocytes in mice is negligible, compared with that of spermatogonia, and mature and maturing oocytes in mice have a mutation rate no greater than 0.44 times that found in spermatogonia. We do not know which of these two classes of oocytes would have a mutational response more similar to that of arrested oocytes in women. To incorporate this range of uncertainty into our risk estimate for the combined effect of irradiation of both sexes, we have simply kept the lower limit of our estimate the same as it was (assuming a negligible mutation frequency in resting oocytes) and multiplied the upper limit by 1.44 (assuming the maximal estimate of the mutation frequency in mature and maturing oocytes). This gives an estimate of 5-65 induced serious disorders per million liveborn as the first generation expression, after exposure of the entire population to 1 rem per generation."

702. For estimating the risk from the induction of reciprocal translocations, the UNSCEAR in its 1977 report used the marmoset and human cytogenetic data as the basis and arrived at an estimate of between 2 and 10 congenitally malformed children per million births per 10^-2 Gy of low-LET irradiation of males. Briefly, the calculations were the following: 7 x 10^-4 (rate of translocation induction; spermatocyte data) x 0.25 (multiplication factor to get the rate for heritable translocations in the progeny) x 0.1 or 0.5 (multiplication factors to account for dose-rate effect after chronic gamma and low dose rate x rays, respectively) x 2 (multiplication factor to estimate the rate of production of unbalanced products of reciprocal translocations) x 6% (the proportion of unbalanced products that was assumed to give rise to viable, but congenitally malformed progeny). The risk for the irradiation of human females was considered to be small, but no quantitative estimate was given.

703. For the BEIR Committee's calculations of risks for irradiated males, the starting point was the same cytogenetic data as those used by UNSCEAR. The estimate of risks (see Table 41) was also nearly the same, but the method of calculations was different. Some of the salient aspects of the calculations and the rationale behind them can be summarized as follows.

704. In mice, the ratio of the observed incidence of partial sterility to that calculated on the basis of the incidence of multivalents in primary spermatocytes was about 1.2 for 500 R and higher exposures, but was 1:1 at 150 R, for reasons yet unknown. To take into account the above uncertainty, the BEIR Committee assumed an overall ratio of 1:1.5. Thus from the rate of 7.7 x 10^-4 per R (spermatocyte data; note that the UNSCEAR used a figure of 7 x 10^-4) the "rate of potentially transmissible rearrangements in spermatocytes" was estimated as 4.7 x 10^-4/rem (i.e., two-thirds of 7.7 x 10^-4; the UNSCEAR's calculations imply a ratio of 1:1).

705. To convert the above figure into one that will be applicable at low total doses or at low dose rates, the BEIR Committee used a reduction factor of 2. This figure was arrived at by assuming a quadratic model for translocation induction in mouse spermatogonia, applying it to the published mouse data of Searle et al. ([S125]; 600 R; different dose rates), estimating the contribution of the linear and quadratic components to the total yield at an exposure of 100 R (because the human and marmoset data pertain to exposures of 100 R and below) and thus computing the expected dose-rate effect. The resultant rate was 2.3 x 10^-4 per rem.

706. From the above rate and assuming that on the average, alternate segregations occur in 45% of the spermatocytes and that half of the recoveries carry the translocation, the BEIR Committee computed that the probability of transmitting a newly-induced translocation to an offspring will be 5.2 x 10^-5 per rem (i.e., 2.3 x 10^-4 x 0.45 x 0.50). It follows that "the combined expectation for all adjacent segregations" would be 55% of 2.3 x 10^-3 or 1.3 x 10^-3.21 Assuming further that "no more than 5% of all translocations are capable of producing viable aneuploids", the probability of generating this was estimated as 5% of 1.3 x 10^-4 or 6.5 x 10^-4 per rem.

707. The BEIR Committee stated: "... however, we would expect only one of the four kinds of aneuploid segregations to be capable of giving rise to viable zygotes. Taking into account that this might not always be one of the less frequent products, dividing by 4 to accommodate the one-out-of-four expectation gives the figure of 1.6 x 10^-4. Using the order-of-magnitude range of uncertainty ... gives the range 0.5 x 10^-4 to 5 x 10^-4 per rem."22

708. For estimating risks for irradiated females, the BEIR Committee made the same assumption as that used in their 1972 report, namely, that the rate of induction of reciprocal translocations in females may be the same as that in males. "Thus, the expected frequency of viable aneuploids for both sexes is assumed to range from 1 x 10^-6 to 10 x 10^-4 per rem." The BEIR Committee also used "an alternative and independent approach based on litter-size reduction observed after acute irradiation of mouse germ cells" and pointed out that "the upper limit of 10 x 10^-4 for both sexes combined may be an overestimate, and that the true value could indeed be near to zero".

709. In conclusion, therefore, it can be stated that there is no major disagreement between the quantitative values presented in the 1977 UNSCEAR report and in the 1980 BEIR report, although some of the methods used are not the same.

D. CURRENT RISK ASSESSMENTS OF THE COMMITTEE

1. Direct method

(a) Mutational damage

710. As mentioned earlier, in its 1977 report, the Committee estimated genetic risks using a direct and a doubling dose method. With the former (and using the data on the induction of skeletal mutations in male mice as a basis), it was estimated that the risk from the induction of mutations (which will be expressed in the first generation progeny as serious genetic diseases, handicaps and disabilities) is of the order of 2000 cases per million births per Gy of paternal, low-LET, low dose or low dose rate irradiation. The Committee considers that the above estimate is still valid.

21 Alternate and adjacent-1 segregations have the same meiotic consequences in translocations, providing there is chiasma formation in at least one of the interstitial segments, which is normally the case (see [S126]). In the absence of adjacent-2 segregation therefore, one-quarter of the sperm produced by spermatocytes heterozygous for a translocation will carry the translocation in balanced form. This is indeed a maximum figure, as stated in the BEIR report.

22 See however Dutrillaux et al. [D26].
711. An independent estimate of risk from the induction of mutations in males can be arrived at using the data on the induction of dominant cataract mutations in male mice following spermatogonial irradiation. These data discussed in subsection 11.D.8 permit estimates of 12.7 $10^{-7}$R/game (fractionated gamma irradiation; 455 + 455 R, 24 h interval) and 5.0 $10^{-7}$R/game (average estimate based on the data from the two single gamma-ray exposure experiments involving 534 R and 600 R, respectively). These rates however, need first to be converted into those that will reflect the response under low dose, low dose rate irradiation conditions in the mouse, and, secondly, to be transformed into quantities that will express the risk in humans. The performance of these operations rests on the validity of the following two assumptions. Firstly, that the quantitative changes in response of the dominant cataract mutations and of recessive specific locus mutations in mice to changes in dose rate and dose fractionation procedures are similar (and its corollary, that, if the rate under one set of radiation conditions is known, those for other radiation conditions desired can be estimated using the specific locus data). Secondly, that since in man, about 2.7% (20/736) of all known and proven dominant mutations are associated with one or another form of cataract [M61], the reciprocal of this (i.e., 100/2.7 = 36.8) can be used in the context of estimating the overall genetic risk from the induction of mutational damage [E20].

712. The relevant calculations are the following:
(a) Expected rate of induction of cataract mutations at low doses or low dose-rates of low-LET irradiation:
(i) From the data in exposure fractionation study:

$$
\frac{12.6 \times 10^{-7}}{1.2 \times 3} = 3.5 \times 10^{-7}
$$

(the factor 1.2 comes from the enhancement effect observed due to fractionation, in concurrent specific locus studies; the factor 3 is the dose rate reduction factor);

(ii) From the data on single exposures:

$$
\frac{5 \times 10^{-7}}{3} = 1.67 \times 10^{-7}
$$

(the factor 3 is the same as in (i) above).

The average of these two estimates (weighted by the number of mutants in the fractionation and single exposure series) is 2.6 $10^{-7}$.

(b) Overall risk from the induction of dominant mutations causing serious effects in the first generation progeny:

$$
2.6 \times 10^{-7} \times 36.8 \approx 10 \times 10^{-6}
$$

(the factor 36.8 is the same as that mentioned in the preceding paragraph). In other words, about 10 individuals per million born will be affected by one or another kind of clinically important serious genetic disease (of induced mutational origin) per $10^{-2}$ Gy of paternal, low dose rate or low dose, low-LET irradiation (or 1000 individuals affected per million born per Gy of irradiation under the stated conditions). It is worth pointing out that in these calculations, the multiplication factor 2 and the division factor 2 used in similar computations with the skeletal mutations to take into account ease of diagnosis and severity of effects, respectively (see Section VII.A), have not been employed.

713. It is clear that the above estimate of 1000 cases per million per Gy of paternal irradiation is similar to that derived using the data on the induction of dominant skeletal mutations in mice. The finding that the estimates derived from two different sets of mouse data and using different correction factors are similar strengthens the earlier conclusions of the Committee. However, it bears reiterating here that all these estimates involve a number of assumptions and that these estimates merely reflect the current status of knowledge and may be subject to revision at some future date.

714. Since there are no experimental data on the induction of either skeletal or cataract mutations in female mice, it is not possible to use a similar approach to estimate the risk associated with the irradiation of human females. However, the probable magnitude of risk to the latter can be derived in a very indirect manner by comparing the specific locus mutation rate estimates for spermatogonia and mature (and maturing) oocytes after low-level low-LET irradiation. As the Committee stated in its 1977 report "...the four rates for low-level irradiation of mature and maturing oocytes estimated by Russell are only 0.17, 0.27, 0.33 and 0.44 times as effective, and only in the highest of these is the induced rate in oocytes significantly above the control rate. Thus the ratio of effectiveness to the spermatogonial mutation rate could be zero".

715. If, for the sake of argument, it is assumed that these ratios will hold also for the induction of mutations having dominant effects; and if it is accepted that the mutational response of the human oocyte will be more similar to that of mature and maturing mouse oocytes for low-level irradiation; then the risk from irradiation of human females can be estimated to be in the range from 0 to 0.44 of that from irradiation of males. In other words, irradiation of human females may entail a risk of producing between 0 and 9 affected children per million births per $10^{-2}$ Gy or between 0 and 900 affected children per 108 births per Gy of low-LET low level radiation.

716. It should be stressed that the computation of this estimate is not meant to suggest that a new risk from irradiation of females has been uncovered, but rather, to take into account the possibility that the mutational sensitivity of the immature human oocyte may not be as low as that of the immature mouse oocyte. Thus the earlier conclusion of the Committee is still valid, namely that: "...even in the event that the human immature arrested oocyte does not respond like the mouse arrested oocyte, but more like the most sensitive stages in the mouse, it seems likely that the genetic hazard of radiation in the female will still be less than in the male". This applies to low dose or low dose rate low-LET irradiation.

717. As discussed in chapter I of this Annex, the thesis that the impact of spontaneous mutations in man that are classified as recessives and which are null mutations in a molecular sense (i.e., those which cause an absence or near-absence of enzyme activity) on human health may be currently underestimated remains speculative. Nonetheless the Committee takes note of this point, but wishes to point out that at present there is no way to translate this concern into meaningful figures. It believes that the risk from the induction of mutations having dominant effects in the progeny far outweighs that from the induction of recessive mutations per se.
718. Turning now to possible risks from the induction of minor (polygenic) mutations, the UNSCEAR 1972 report [UR] stated the difficulties involved in making a risk estimate for this kind of mutations as follows: "... Experiments with Drosophila show that mutation resulting in minor deleterious effects grossly outnumber those with severe effects. The calculations... do not take into account this class of mutations which lead to minor disability and disease... this bias of the greater frequency of occurrence of these mutations, their total effect in terms of genetic burden to the population could be greater than that of a smaller number of relatively more serious conditions... There is, however, no way at present to assess their contribution to the genetic burden of man". In the 1977 report, the above general conclusion was implied (although not explicitly stated) and no risk estimates for this class of mutations were made. The situation has not changed in the meantime and therefore, the Committee does not see a need to alter the above point of view. (For recent reviews on the effects of spontaneously-occurring and radiation-induced polygenic mutations on population fitness in Drosophila, see [S127, M68].)

719. In its 1972 and 1980 reports, the BEIR Committee expressed a similar opinion. The 1972 report stated the situation as follows: "... Perhaps the major reservation that we have about our estimates is the failure to take adequately into account mutations that have very mild effects... this is the most frequent class of mutations in Drosophila and because they persist longer in the population than those with more drastic effects, each mutant affects a correspondingly larger number of persons... Perhaps the human counterparts of these mutations, in addition to causing a slight reduction in life expectancy, are responsible for greater susceptibility to disease, impaired physical or mental vigor, or a slight malformation of some organ... Despite a concern for this effect, we shall not attempt to estimate it quantitatively. At least in Drosophila, the evidence is now good that this class of mutations is relatively less frequent among radiation-induced mutations than among spontaneous mutations... The empirical experiments on mice argue that such genetic mutations are not making any substantial impact on mouse populations for up to 45 generations of continuous radiation, far longer than we are able to consider in any meaningful way for the human population".

(b) Chromosomal damage

720. As was discussed earlier, the Committee made use of the limited human and marmoset cytogenetic data to give estimates of risk from the induction of reciprocal translocations in males in its 1977 report. The risk was estimated as 2 to 10 cases of congenitally malformed children per million births per 10^-2 Gy (or 200 to 1000 such cases per million births per Gy) of low level, low LET irradiation with the risk assumed to stem primarily from the unbalanced products generated by radiation-induced balanced reciprocal translocations (see Section VII. C for a recapitulation of the procedure used in the calculations).

721. Studies on rhesus monkey spermatogonia using improved techniques have yielded far lower rates of translocations than those previously reported for human and marmoset spermatogonia. Since there is no a priori reason to assume that human spermatogonial sensitivity is similar to those of the marmoset and since the possibility exists that human spermatogonia may manifest a pattern similar to that of the rhesus monkey, one can use the rhesus monkey data to define a probable lower limit of risk; this procedure will not materially alter the Committee's earlier assessments as long as it is assumed that the sensitivity of human spermatogonia may lie anywhere between the limits defined by those of rhesus monkey and marmoset.

722. If this line of reasoning is accepted, then correction factors similar to those used in the 1977 report can be used to arrive at an estimate of risk as follows (all rates are per 10^-2 Gy):

(a) Rate of induction in rhesus monkey spermatogonia; cytogenetic data 0.86 10^-4
(b) Rate of induction that relates to recoverable translocations in the F1 progeny [divide (a) by 4] 0.215 10^-4
(c) Rate after low dose rate x rays [divide (b) by 2] based on mouse cytogenetic observations 0.1075 10^-4
(d) Rate after chronic gamma-irradiation [divide (b) by 10] based on mouse cytogenetic observations 0.022 10^-4
(e) Expected rate of unbalanced products: [multiply (c) and (d) by 2]: for (c) 0.215 10^-4 for (d) 0.043 10^-4
(f) Expected frequency of congenitally malformed children in the F1 assuming that about 6% of unbalanced products [item (e) above] contribute to this: low dose rate x rays 1.3 10^-8 chronic gamma irradiation -0.3 10^-8.

Thus, on the basis of the rhesus monkey data, one can estimate that the risk of producing congenitally malformed children (as a consequence of the induction of balanced reciprocal translocations in the fathers) is between 0.3 per 10^4 and 1.3 per 10^6 per 10^-2 Gy (or between 30 and 130 per 10^6 per Gy) of paternal low level irradiation. Taking into account all the primate data, it can be estimated that there will be between about 30 and 1000 cases of abnormal children per million progeny per Gy of paternal low level irradiation, stemming from the unbalanced products of radiation-induced balanced reciprocal translocations.

723. Although the risk estimated above is based entirely on unbalanced products of balanced reciprocal translocations, the Committee takes note of the observations in humans (chapter I) and in mice (subsection II.D.8) that some balanced reciprocal translocations are associated with dominant phenotypic effects. The risk from these is not, however, ignored: it forms part of the total risk estimated for mutational events with dominant effects. Thus, some of the dominant skeletal mutations are associated with, and probably caused by, balanced reciprocal translocations. The basis for dominant effects of balanced reciprocal translocations has not been elucidated, although at least two formal possibilities can be envisaged: loss of chromosome material at the site of chromosome breakage, perhaps too small to be detected, and position effect. In humans, the documented cases of position effects involve only the X chromosome [D16, T26] and are associated with abnormal phenotypes. In mice, in addition, there is evidence that position effects can also be generated in the case of autosome-autosome translocations.

724. The rate of translocation induction in human females is not known and there are no marmoset or rhesus monkey data. In its 1977 report, the Committee
stated that "... the data for mouse females show that in maturing oocytes exposed to acute x-irradiation, the rate is 0.16 10−4/gamete/rad ... although there is no direct evidence on the response of the immature oocytes stages to the induction of translocations at low doses and dose rates, the data on specific locus mutations and on X-chromosome losses strongly support the view that the rate for translocations is also likely to be low, but no quantitative estimate can be given"

725. It is now possible to make further arguments (necessarily indirect) and calculations to show that the above statement is probably correct for low dose rate gamma irradiation. One can start with either of the following two assumptions: for radiation conditions applicable to humans, the immature human oocyte will respond in a manner similar to the mature and maturing mouse oocyte, and the immature human oocyte will respond in a manner similar to that of the immature mouse oocytes. In the latter case, the risk is negligible or zero. The following discussion focuses on the consequences if the first assumption is correct.

726. In mice, as discussed earlier, maturing and mature oocytes are only one-half as sensitive as spermatogonia to the induction of heritable translocations. There is a pronounced dose-rate effect (subsection II.B.4) for the induction of chromatin in the changes in maturing oocytes. If these findings are applicable to humans, marmosets and rhesus monkeys, the following two rates of heritable translocation induction can be computed for high dose rate low-LET irradiation (all rates per 10−2 Gy): 0.875 10−4 (marmoset and human data base; 0.5 0.25 7 10−4) and 0.108 10−4 (rhesus monkey data base; 0.5 0.25 0.86 10−4). At low dose rates, these rates will be an order of magnitude lower (i.e., 0.0075 10−4 and 0.0108 10−4).

727. Since heritable translocations recovered from irradiated oocytes are induced as chromatid interchanges, a gamete from an affected oocyte would be six times more likely to have an unbalanced form of the translocation than a balanced one (see references [S128, S135]). In other words, the rates for unbalanced products will be six times those quoted above (i.e., 0.5275 10−4 per 10−2 Gy and 0.0648 10−4 per 10−2 Gy). Assuming as before that about 6% of the unbalanced products will result in congenitally malformed children, it can be estimated that there will be 3 children per million who will be affected per 10−2 Gy (or 300 per million per Gy) (marmoset + human data base) or 4 affected children per 10 million births per 10−2 Gy (or 40 per million per Gy) (rhesus monkey data base) of maternal, low-LET, low level irradiation. In either case, it is clear that the risks are low for irradiated human females. It is worth reiterating here that if the sensitivity of the human immature oocytes is similar to that of immature mouse oocytes, the risk will be close to zero.

728. With respect to structural aberrations other than reciprocal translocations, the Committee's earlier view that the risk from their induction is probably small (since most of such changes may act too early to constitute a real hazard in liveborns) is still valid. It is known that deficiencies are induced and no doubt some of these will contribute to dominant effects. The risk for these, like that for balanced reciprocal translocations, is included in the scoring of mutational events with dominant effects. Likewise, the Committee's earlier conclusion (based on mouse data) that the risk from the induction of sex-chromosome losses in males is very low or nil is still valid. For chronic gamma irradiation of maturing oocytes of mouse females, L.B. Russell [R30] has estimated (from the data of W.L. Russell and colleagues) an induction rate of 5 10−6/R; for irradiation of immature oocytes, the induction rate is zero. These findings would suggest that if the human oocytes respond like those of the mouse, the risk is probably no higher than 5 10−6/R (or about 500 10−6/Gy) for irradiation of females. Similarly, the Committee's earlier conclusion that the risk from the induction of nondisjunction (leading to viable trisomies) is small, although it cannot be quantified at present, still stands.

729. The "expected rates" of induction of different kinds of genetic damage (derived from the relevant data discussed in this section) are summarized in Tables 42 and 43.

2. Doubling dose method

730. The estimates of risk arrived at by the Committee using the doubling dose method are given in Table 44. They are essentially the same as those given in their 1977 report, except for three changes: (a) the current estimates are based on an assumed exposure of 1 Gy/generation (instead of an assumed exposure of 10−2 Gy/generation, as was the case in the 1977 report) of low dose rate, low-LET irradiation; (b) on the basis of the calculations of Childs [C68] for dominant and X-linked diseases, discussed earlier, the Committee has now assumed that the first generation increment of these diseases is 15% of that at equilibrium, instead of the 20% figure assumed in the 1977 report; and (c) the Committee has refrained from applying the doubling dose of 1 Gy to the overall incidence of chromosomal diseases in order to estimate the increment which would be expected to result from irradiation. Instead, it has applied the doubling dose only to the structural component of these diseases, for the reasons discussed below.

731. Chromosomal diseases are divided into two very distinct components, namely, those based on structural and those based on numerical changes. Structural changes in the form of reciprocal translocations were included in the mutational end-points from which an overall doubling dose of 1 Gy for low dose, low-LET irradiation was calculated. Therefore, it seems appropriate to apply the same value to the structural anomalies in Table 44. However, data on trisomies which result from the process of non-disjunction rather than chromosome breakage, were not used in doubling dose calculations and the results of more recent experiments do not permit a doubling dose for this category to be estimated with any confidence. No experiments have been carried out under chronic exposures and no clear-cut dose-response relationship has been obtained under acute exposures. Moreover, there are complicating factors like maternal age which lead to considerable heterogeneity of response. Therefore, no attempt has been made to estimate the likely effect of 1 Gy/generation on the incidence of numerical anomalies. This effect is likely to be very small.

732. For the purpose of doubling dose calculations, the incidence of diseases stemming from structural aberrations of chromosomes has been derived from the figures for structural aneuploids given in Table 2 of this Annex. However, aneuploid Robertsonian translocations have been omitted because of the evidence that these are not induced by radiation (see text); the
"others" category has also been omitted because they are mainly mosaics and therefore probably result from events in early development rather than in either parent. For the same reasons, mosaics have also been omitted from calculations of the incidence of numerical anomalies.

733. Other data and assumptions that are now used to derive risk estimates are the same as those used in the 1977 report. Among these are the following: the use of a doubling dose of 1 Gy to estimate risks at low doses of low dose rate, low-LET irradiation; the assumption of the same incidence figures for the different classes of genetic diseases as those used earlier (1% dominant and X-linked diseases); 0.25% recessive diseases, including those maintained by heterozygous advantage, but this inclusion does not affect the risk estimate; 0.34% chromosomal diseases and 9% diseases of complex aetiology; the assumption that under conditions of continuous exposure to low level low-LET irradiation, the incidence of diseases in the population will reach a new equilibrium with an elevated incidence rate; and the assumption that the equilibrium frequency, the rate of approach to equilibrium and the expected effects in the first generation will be dependent on the kind of disease under consideration.

734. It should be noted that the genetic risk estimates given in Tables 43 and 44 refer to gonadal radiation exposures before or during the reproductive period, i.e., all the dose is genetically significant. When dealing with population exposures, it is sufficient to assume for the purposes of calculation that the child expectancy per parent is 1.0 until the mean age at conception and zero thereafter. Thus, if the mean age at conception is 30 years, then the genetically significant dose will be that received by the gonadal germ cells up to the age of 30. If the mean life expectancy in the population is 70 years, then the genetically significant dose will be 3/7 of the mean lifetime gonadal dose.

3. Index of harm, genetic detriment and the impact of genetic disease

(a) Introduction

735. The quantitative figures of risk arrived at earlier (Tables 43 and 44) refer to expected numbers of cases of "serious genetic disease" due to radiation-induced mutations and chromosome aberrations in the progeny of those exposed to irradiation. The term "serious genetic disease" used in this context connotes ill-health, handicap or disability of genetic origin which can set in at any time from birth onwards: it does not, however, discriminate between the impact of these diseases on the individual, family, society or health-care facilities, to mention only a few. As Newcombe has stressed [55], "... such numbers are poor indicators of harm if one lacks a satisfactory measure of the spectrum of severities among the various individuals affected by the hereditary conditions". For instance, no one doubts that Huntington's chorea and Down's syndrome are serious genetic diseases, but yet they are different: Huntington's chorea is a disease in which there is a gradual but very serious degeneration of the central nervous system with onset during the third to fifth decades of life. Down's syndrome children suffer from a multiplicity of problems from birth onwards.

736. What is obviously needed are some objective and quantifiable indices of severity such as years of life lost due to the disease, the relative durations of hospitalization or medical care needed, etc., which can, at least in principle, be used to "weight" the different diseases to arrive at an overall estimate of genetic detriment. Only on the basis of such an estimate (or a group of estimates, depending on the criteria used) can one make meaningful comparisons between, and realistic appraisals of (i) the impact of diseases of different genetic aetiologies and (ii) the relative contribution of somatic and genetic effects to human ill-health. This premise is valid for both spontaneously-occurring and induced effects.

737. These considerations would suggest that, in the context of comparing genetic and somatic effects of radiation, equating the number of cases of induced genetic disease per unit amount of radiation with the number of cancer deaths (and then adding the two together as a measure of total harm or detriment) is neither wholly satisfactory nor entirely adequate. An example will make this point clear: if a fatal induced cancer involves, say, 10-12 years of loss of life and if the average age at death from what are classified as major genetic defects were at age 15 (i.e., with about 60 years of loss of life expectation), there would immediately be a factor of 4 or 5 greater average detriment for each major genetic defect than for each fatal cancer, as judged by only life loss, and apart from the difference in disability during life.

(b) Indices of harm

738. A recent ICRP document [6] considered in detail the problems involved in developing indices of harm from the standpoint of recommending appropriate limits for any occupational or other exposure to radiation and the assessment of the safety of an occupation involving such an exposure and comparing it with the safety of other occupations. In that document, both the limitations and utility of certain criteria such as fatality and mean loss of life, various occupational injuries and their severity (expressed for instance as the total number of working days lost) were dealt with and compared with similar consequences of somatic and genetic effects of radiation exposures. It suggested that the index of time lost (an integrated measure taking into account all these criteria) from a full and normal working life (expressed as man-years per year per 1000 employed) is a possible approach, despite a number of limitations.

(c) Genetic detriment

739. The reasons why, in spite of its importance, the problem of developing adequate measures of genetic detriment has been largely neglected thus far, have been succinctly stated by Trimble and Smith [2] and can be summarized as follows. It is difficult to define parameters that could be considered valid measures of overall burden; it is equally difficult to amass large volumes of objective data covering the whole life span of both diseased and normal individuals, and such research is still generally thought to be scientifically unrewarding. Notwithstanding these difficulties, a few attempts have been made to develop an index of harm for genetic diseases. Although all these attempts concern spontaneously-occurring genetic diseases, the remainder of this section will be devoted to a discussion of these, if only to illustrate the methodology that could
eventually be used to assess the genetic effects of radiation.

740. Several tangible criteria have been used to assess and quantify the actual burdens imposed by genetic diseases on the individual, the society, and public health facilities. These include mortality (and consequent reductions in life expectancy), number of days of hospitalization, frequency of hospital readmission, etc. These do not include however entities such as the reduction in the quality of life for the carrier of a given genetic disease nor does it include the anguish experienced by the parents of a child with a genetic defect. There is no doubt that these are important, but they do not readily lend themselves to quantification.

\[ e^* = \frac{100000 \frac{1}{\bar{y}_{DS}} - 1}{100000 - 1_{DS}} \]  

Substituting in equation (1) gives

\[ YLL_{DS} = 1_{DS} (e^* - \bar{y}_{DS}) + \frac{1_{DS} (e^* - \bar{y}_{DS})}{100000 - 1_{DS}} \]  

744. The data and assumptions used are the following: the incidence figure for Down's syndrome at birth estimated by Carter et al. [C48] of 1 in 660 in England is a reliable one and can be considered applicable for the United States; although the sex distribution at birth appears slightly to favour the males, with complete ascertainment at birth, the female and male neonates with Down's syndrome would be of equal numbers; and the data from two large studies of mortality, one an American and the other a Danish, can be used; in the first, Fabia et al. [F20] have presented sex-specific life tables up to 10 years of age, based on all Down's syndrome children born alive in Massachusetts from 1950 through 1966 (a total of 2421 cases). In the second, Oster et al. [O19] have presented similar tables from 5 to 80 years of age for all the Down's syndrome cases (526 cases) born in a certain area of Denmark in 1949.

745. From these data, Jones calculated that the average age at death for Down's syndrome males is 35.7 years and for females, 35.5 years. In 1970, the life expectancy at birth in the general United States population was 67.1 years for males and 74.8 for females [N19]. Applying equation (2), he arrived at a YLLDS figure of 5364.2 years per 100 000 persons born alive, or a little more than 53.5 years for every 1000 livebirths.

746. With diseases such as Huntington's chorea, the situation is somewhat different. In these cases, having the genotype (but not the disease) is usually not a risk-altering state. Since the onset is often gradual, fixing a point in time in the natural history of the disease with delayed onset (where the risks to a patient's life first differ from the risks for other people) is difficult. It can conservatively be assumed that until onset as conventionally defined for that disease, the person's life is subject to the same forces of decrement as others. Account must also be taken of the fact that many people with the genotype for the disease never develop it because they die before the onset. In a disease with delayed onset, the incidence at birth of the underlying genotype is always higher than lifetime incidence, i.e., the number of persons relative to (say) 100 000 born who will develop the disease at some time in their lives. In calculating years of life lost, it is the latter number (lifetime incidence) which is important, since under the assumption used, the lives of people who die before onset have not been shortened by the genetic disease for which they are at risk [J13].

747. Jones [J13] has shown that lifetime incidence, although rarely reported in the literature as such, can be estimated from the relationship

\[ I_g = \frac{B_g}{S_g} \]  

where \( I_g \) represents lifetime incidence per 100 000 born; \( B_g \) is the number of persons in the life-table population affected at a given time; and \( S_g \) is the average duration of disease g. In other words, incidence = prevalence x duration. He has also shown that once \( I_g \) is determined,
the remainder of the calculations is essentially the same as for a disease which manifests itself at birth (e.g., Down's syndrome considered earlier). The equation is

\[ \text{YLL}_g = \sum_{x \in \mathbb{H}} (p_x \xi_g) (\xi_g - \bar{s}_g) = \frac{\xi_g}{\bar{s}_g} (\xi_g - \bar{s}_g) \]

years per 100 000 born

(5)

where \( p_x \) is the proportion of \( 1 \) with onset at age \( x \); and \( \bar{s}_{g,x} \) is the expected duration of illness for a person with onset at age \( x \).

748. The data used by Jones are those published by Reed and Chandler [568] for Michigan for which the prevalence was estimated by Reed and Chandler as 4.1 per 100 000; to obtain this figure, these authors determined the number of chorlications living in the Lower Peninsula of Michigan on April 1, 1940 and divided this number by the total Lower Peninsula population on that day. Jones however based his calculations on the national 1970 life-table population which contained more chorlications per 100 000 than reported by Reed and Chandler. Weighting the age-specific prevalences from the Michigan study according to the 1970 age-distribution yielded an overall prevalence in the 1970 life-table population of 4.8 chorlications per 100 000. The 1970 life-table population numbered 7 085 472 persons, and the total number of chorlications in the study population was 338.

749. In the Reed and Chandler study, the average duration of illness was essentially the same for both sexes: 15.85 years. Using equation (4), the number of men and women per 50 000 born who became choricle at some time in their lives can be estimated as 169/15.85 = 10.7 persons; for both sexes together, the lifetime incidence is 21.3 persons per 100 000. Examination of the age distribution at onset revealed that the average life expectancy at onset was 36.1 years for males and 41.2 years for females. Using equation (5)

\[ \text{YLL}_{HD} = 1_p (\xi_g - \bar{s}_g) = 10.7(36.1 - 15.85) = 216.7 \text{ years} \]

for every 50 000 males and 10.7 (41.2 - 15.85) = 271.2 years for every 50 000 female births. For both sexes together, a little less than 5 years of life are lost due to HD for every 1000 persons born.

750. A comparison of the above figure with that obtained for Down's syndrome will reveal that the effect is about an order of magnitude higher for the latter when the criterion of life loss is used. In other words, the amount of genetic detriment associated with Down's syndrome is much higher than that with Huntington's disease, partly owing to the seven times higher incidence of Down's syndrome. It bears reiterating that the detriment measured here pertains to life loss only and does not include the personal or family hardship and the trauma associated with having a genetic disease.

(ii) Does a given kind of genetic disease lead more frequently to early mortality? The results of the British Columbia study

751. Thus far, the most extensive and perhaps the most illuminating studies, from the standpoint of the assessment of the relative impacts of different classes of spontaneously-occurring genetic diseases, are those by Trimble and Smith [22] and by Newcombe [55]. Both these represent an extension of the earlier work by Trimble and Doughty [11] on the incidence of genetic disease in the Canadian province of British Columbia with a current population of over two million people. Using automated record linkage, ill-health records of various kinds have been linked to the records of all births (approximately 800 000). Trimble and Smith estimate that nearly 90% of all potentially linkable pairs of ill-health and birth records were successfully brought together and linked. The specific examples, to be discussed below, were obtained using the health histories of 835 774 children who were born in the province between 1946 and 1970, including the histories for 370 children with known single-gene dominant disorders, 559 with identified recessive diseases. 928 with chromosomal diseases of which 43 were not classified as chromosomal diseases and the remainder with autosomal disorders, primarily Down's syndrome) and 14 460 with irregularly inherited congenital malformations.

752. One of the questions asked was whether a given kind of disease leads more frequently to early mortality. As Table 45 shows, within the first year of life, approximately 2.5% of all liveborn children die from one cause or another: this value is about 4 times higher for children with dominant or recessive disorders. The second through fifth years of life compared to the same risk for infant mortality: for surviving children with recessive diseases, there is an almost 20-fold increased risk of death in the second and fifth years of life: children with congenital malformations, however, appear to have a fairly constant, 4- to 6-fold higher risk of dying at all ages up to five years, when compared to all livebirths; and by ages 4 and 5, children with single-gene disorders, especially those with recessive diseases, appear to have even greater absolute risks of dying compared to children born with irregularly inherited disorders.

753. Figure III (taken from the paper of Newcombe [55]) compares the age-specific cumulative mortality among cases in different categories of genetic diseases over the first nineteen years of life; mortality is expressed as a factor by which it exceeds the expected numbers of deaths based on all births in the same years. It can be readily seen, with this criterion, that recessive diseases are associated with the highest severity; that diseases classified under the headings "multifactorial", "environmentally caused" and "unknown aetiology" are associated with the least severity; and that the remaining classes fall in between.

754. As Newcombe [55] cautions, these different degrees of severity apply, however, only to the cases that actually got into the different registries and are clearly not applicable to those manifestations that were too mild to be registered as handicapping conditions. Thus for instance, whereas the registration of Down's syndrome in the British Columbia registries can be considered complete, the same is not true of other
chromosomal anomalies. Newcombe has estimated that about two-thirds of the total cases of chromosomal anomalies are not registered in the above registries. The exclusions, however, are of the less severe traits. In assessing the public health impact of these diseases, one should clearly not take data on degrees of severity as derived from cases that have attracted special attention and then assume that these severities are typical of the total number of cases as derived from the surveys that have aimed at complete ascertainment.

755. While mortality is a useful measure of severity and data on this are more readily obtainable, one should not over-emphasize its importance. For instance, for chromosomal diseases, mortality per se is perhaps one of the least useful indicators of severity and this would be particularly true of Down's syndrome which may result in prolonged stays in institutions from an early age. In such circumstances the burden to society is perhaps inversely related to mortality. The same is true of several single gene diseases which have a late onset in life and of sex-chromosomal anomalies which tend to be mild and to escape notice until after puberty.

(iii) Overall estimates of lost life and impaired life for spontaneously-arising genetic diseases

756. Recently, Carter [C31, C69] has provided some rough estimates (Tables 46-49) of the average disability caused and the average length of life lost by the more common genetically determined diseases in a developed country. Carter did not give similar estimates for irregularly-inherited diseases, but the Committee has made some crude estimates for these diseases (see Table 50) taking into account the list of Trimble and Doughty [T1], but wishes to caution that there may be considerable errors in these estimates. In considering the information presented for single-gene dominants, X-linked recessives and autosomal recessives (Tables 46-49), the following points made by Carter are worthy of note.

757. The estimates of birth frequency (equivalent to the life-time incidence figures used by Jones [J13, J14]) given in the above tables are mostly those given in review articles by Carter [C49, C50]. They are derived from the prevalence estimates by multiplying the latter by the average duration of life in the population (assumed to be 70 years) divided by the average duration of clinical illness. The birth frequency estimates are underestimates, since these do not include those who die from other causes before the disease becomes clinically manifest. However, in developed countries, this will usually be a small proportion of the whole, and in them, the genetic disorder cannot be said to have caused lost years of life. Three chronic dominant neurological disorders have been added to those quoted in Carter [C50]. Estimates of their prevalence are variable [S129] but they are not uncommon disorders in neurological practice.

758. The estimates of age of onset, duration of clinical illness and age at death are reasonably well established, though in debilitating disorders, where intercurrent infection is the usual final cause of death, modern antibiotics are substantially prolonging life. The estimates of average degrees of disability are inevitably somewhat arbitrary and subjective. The rough guide has been limitation of working capacity in adult life and/or, in childhood, educability. The life-long disability of congenital blindness is somewhat different from a slowly progressive disorder such as dominant cerebellar ataxia, which at first causes little disability but, towards the end, is totally incapacitating; or from neurofibromatosis, which causes little disability in some patients but severe disability in others. While all estimates are approximate, they are perhaps useful for comparison with similar estimates for radiation-induced neoplasms.

759. An examination of the summary Table 50 will reveal that, depending on the index of detriment used, the ranking of severities varies (columns 4 and 5 in Table 50). Thus in terms of years of impaired life, the rank order is: chromosomal diseases > X-linked diseases > recessive diseases > dominant diseases > irregularly inherited. If years of life lost is taken as the index of detriment, then the rank order becomes: recessive diseases > irregularly inherited diseases > X-linked diseases > chromosomal diseases > dominant diseases. The ranking for the degree of impairment is: recessive diseases > chromosomal diseases > X-linked diseases > dominant diseases. Finally, for impaired life weighted for degree of impairment the rank order is: recessive diseases > chromosomal diseases > X-linked diseases > dominant diseases. The finding that dominant diseases as a whole rank lower than others is not unexpected; nearly one-half of all dominant diseases included in the calculations have a late onset (giving unimpaired life durations of 30 years or more) whereas a sizeable proportion of diseases belonging to the other categories have an onset in childhood.

760. The last two columns in Table 50 provide some rough idea of the social impact in terms of impaired or lost life per 106 births. As can be seen, the rankings are now different. On the whole, spontaneously-arising genetic diseases account for about 2 300 000 years of impaired life per 106 births and about 3 000 000 years of life lost per 106 births (the assumed average life expectancy at birth for the general population is 70 years or 70 106 per 106 births).
761. A number of investigators have used utilization of hospital services as a rough measure of the degree of the detriment associated with genetic diseases (e.g., [C5, D17, H2, P32, S85]). In these studies, medical charts of the patients were examined for given periods, the patients subdivided into different categories and data on the number of admissions, length of hospitalization (and in some cases, costs), etc., were collated. Since the diagnostic criteria varied in the different studies, precise comparisons are not possible. The study of Hall et al. [H2] is illustrative of the principal aspects and will be discussed below.

762. This study pertains to cases admitted to the Children’s Orthopaedic Hospital and Medical Centre (COHMC) in Seattle area. 4115 medical charts were examined and the patients were assigned to one of five categories which were:

- (a) Clearly genetic disorders;
- (b) Multifactorial/polygenic conditions;
- (c) Development anomalies;
- (d) Familial disorders;
- (e) Non-genetic disorders.

Each patient was assigned to only one category, irrespective of the number of admissions. The results of the analyses showed that:

- (i) Of all admissions, 4.5% had clearly genetic disorders (0.6% chromosomal, 1.2% autosomal dominant, 2.2% autosomal recessive, 0.5% X-linked recessives), 22.1% had multifactorial/polygenic conditions, 13.6% developmental anomalies, 13.2% familial disorders and 46.6% non-genetic disorders;
- (ii) Patients with clearly genetic disorders (category a) had an average of 5.3 admissions as compared to 1.3 for patients with non-genetic conditions;
- (iii) Of “genetic” patients (category a), 12.8% had more than 20 admissions as compared to only 1.2% for all patients studied;
- (iv) The length of hospitalization for “genetic” patients was 3.4 days as compared to 2.5 days for non-genetic patients;
- (v) In terms of general costs, the “genetic” patients (and their families) incurred more expenses than others.

763. The frequency of genetic diseases among hospital admissions in the above study is compared with similar data from some other studies in Tables 51 and 52. It can be seen that the results are roughly similar, except for some of the entries in the last row. Penchassadeh [P32] points out that this difference is due to several factors among which the most important is the persistent burden of environmental infectious and nutritional diseases in the paediatric age population in Venezuela.

764. In the British Columbia study of Trimble and Smith [T2] mentioned earlier, data on the utilization of hospital services by individuals with different genetic conditions were also collected. These are summarized in Tables 53 and 54. Excluded from the Tables are hospitalizations due to common childhood disorders such as respiratory and infective diseases. It can be seen that for 1000 livebirths, there are some 200 hospital admissions in the first year of life, dropping to about one-third of this figure by the second year and to about one-fifth during the fifth year of life; that children with dominant or recessive diseases or congenital malformations are, on the average, admitted to hospitals 5–7 times more often up to age 1, with the relative risk gradually increasing with age, except for those with congenital malformations; that with respect to hospital stays, considering all livebirths, there are over 200 days of hospitalization per 100 children during the first year of life and this figure decreases to about one-fifth by age 5; that children with dominant diseases account for 9–13 times as much in-patient hospital usage per capita during the first year of life and this value increases drastically for those who survive this period; that for children with recessive disorders, the amount of hospital usage per affected child is 12–24 times greater than for all livebirths, and these children have average lengths of stay per admission per affected child of 13–19 days during the first year of life; and that children with congenital malformations spend consistently slightly less time, on average, in hospital than those with either dominant or recessive diseases, but they have a 7–11 times greater use of in-patient services per capita than normal children. It is worth pointing out that these figures are qualitatively similar to those discussed in paragraph 762.

765. In principle, it is possible to use one or more of the indices discussed in the preceding paragraphs to compute the amount of detriment that is likely to result from the estimated induction of genetic diseases by radiation. It hardly needs to be stressed however that such an attempt is beset with considerable uncertainties, as will be outlined below.

766. Firstly, for those diseases for which a proportionality between spontaneous and induction rates was assumed (i.e., single-gene dominants, X-linked diseases and a small proportion of diseases of complex aetiology), it is necessary to make the additional assumption that the spectrum of detriment for “induced” diseases will be similar to that for spontaneous ones. For instance, for dominant diseases, the numerical estimates of risk given in Tables 43 and 44 signify that all disposes (heterozygous as well as late onset) of spontaneous ones. Whereas for spontaneously-arising diseases one has sufficient knowledge of the degrees of severity, this cannot be said to be true for “induced” ones. The reason for this is that the quantitative estimates of risks from the induction of mutations having dominant effects are based on rates of induction estimated from animal experiments and consequently are subject to the limitations inherent in the extrapolation procedure employed.

767. Secondly, spontaneously-arising autosomal recessive diseases are associated with a considerable degree of detriment (see Table 50). Our risk estimates for radiation exposure however, do not include recessive diseases per se, since their incidence is not expected to increase appreciably in the foreseeable future as a result of radiation exposure.

768. Thirdly, the major component of spontaneously-arising chromosomal diseases is constituted by numerical anomalies. However, as discussed earlier, it has not been possible to provide an estimate for the radiation-induction of numerical anomalies and the risk has been assumed to stem primarily from the unbalanced products of induced balanced reciprocal translocations.
769. Finally, for the class of diseases which is numerically most frequent among spontaneously-arising diseases, namely, the irregularly-inherited ones, it is difficult to make the kind of estimates (in terms of impaired life, life loss, etc.) which Carter has made for other classes. The figures for those given in columns 3–7 of Table 50 are no more than crude guesses and may be associated with considerable errors. This limitation doubtless applies also to estimates of detriment associated with radiation-induced irregularly-inherited diseases.

770. In spite of these problems and difficulties, the Committee considers it worthwhile to attempt some estimates of detriment for radiation-induced genetic diseases, if only to illustrate a possible method and to gain some rough idea of the impact of these relative to that for spontaneously-arising ones. Such estimates are given in Table 55. It is worth reiterating that the numerical values are only approximate and must be viewed in the light of the number of reservations mentioned earlier.

771. It may be noted that the numerical figures given in column 2 of Table 55 (induced cases per $10^6$ births) are those from Table 44, but the dominant and X-linked categories are shown separately. Furthermore, following Childs [C68], the first generation incidence for dominant and X-linked diseases is assumed to be 14% and 25%, respectively, of the equilibrium incidence. For chromosomal and irregularly inherited diseases, the figures given in column 2 are the same as those given in Table 44. For impaired life and life loss, the figures used are the same as those given in Table 50, except that for chromosomal diseases, the figures given in Table 49 for autosomal structural aneuploidy are employed.

772. The general conclusions to be drawn from Table 55 can be stated as follows: if a population is exposed to low dose rate, low-LET irradiation at a rate of 1 Gy per generation, the expected increment in genetic disease is of the order of about 2000 cases per $10^6$ births in the first generation; this frequency is about one-seventh of that at equilibrium. These diseases are likely to cause about 50 000 years of impaired life per $10^6$ births and an equal amount of life loss per $10^6$ births in the first generation. At equilibrium, the figures are about 6 to 7 times higher. A comparison of these figures with the magnitude of detriment associated with spontaneously-arising genetic diseases (Table 50) will show that the former are relatively small for the stated radiation conditions. The Committee wishes to stress again that these figures (Tables 50 and 55) are crude, but may be useful in the comparison of detriment associated with spontaneously-arising and radiation-induced cancers.

E. SUMMARY AND CONCLUSIONS

773. In its 1977 report, the Committee made use of both the “direct” and “doubling dose” methods to obtain quantitative estimates of genetic radiation hazards in humans. The main conclusions were the following.

774. Using the direct method, the Committee estimated that following low-LET, low dose rate irradiation of males, there will be about 20 cases of affected progeny per million births per $10^{-2}$ Gy who will suffer from the effects of induced mutations having dominant effects. The data on the induction of dominant skeletal mutations in mice were used to make this estimate. For structural aberrations of chromosomes—predominantly reciprocal translocations—the risk was estimated to lie between 2 and 10 per million livebirths per $10^{-2}$ Gy under similar radiation conditions. The cytogenetic data on radiation-induction of reciprocal translocations in marmoset and human males were used for this purpose.

775. The risk for irradiation of human females, both from the induction of mutations having dominant effects and from the induction of reciprocal translocations was considered low, but no quantitative estimates were given.

776. The risk from the induction of sex-chromosome losses in either sex was also considered low, for the radiation conditions applicable to humans.

777. The risk estimate arrived at using the doubling dose method was that, under conditions of continuous radiation exposure to low-LET, low dose rate irradiation at a rate of $10^{-2}$ Gy/generation, the additional number of cases of genetic disease will be about 63 per million births in the first generation and about three times this frequency at equilibrium (over and above the 105 200 per million births occurring spontaneously). The doubling dose assumed was 1 Gy.

778. Since the publication of the 1977 report, new data have become available. Among these are those which confirm and further document the Committee’s earlier conclusions: those that help to shed light on the validity of the assumptions and tentative conclusions (arrived at on the basis of limited data) or controversial view-points: those that are relevant in a qualitative sense, but which as yet cannot be used in quantitative risk assessments; and those that are of relevance for quantitative risk assessments. These have been briefly reviewed in this chapter. The new data pertain to the induction of dominant cataract mutations in mice and to the induction of reciprocal translocations in the rhesus monkey. Use was made of these data (in addition to those that were used in the UNSCEAR 1977 report) in quantitative hazard evaluations.

779. New publications on quantitative estimation of genetic hazards in humans (those of individual authors and of scientific bodies) have appeared since the UNSCEAR 1977 report. Brief summaries of the main conclusions reached in these are given, in addition to some detailed discussions on the similarities and differences between the conclusions reached by the UNSCEAR in 1977, an ICRP Task Group and the BEIR Committee in its 1980 report. It is pointed out that the conclusions reached by all three scientific bodies are similar and where differences exist, they stem from the different assumptions used (the basic data for all three are the same).

780. The Committee’s current estimates of genetic hazards have also been made using the direct and doubling dose methods. With the former method, the risk from the induction of mutations having dominant effects in the progeny has now been estimated to lie in the range of 1000 – 2000 cases of affected individuals per million born per Gy of low-LET, low dose rate
irradiation of males. For the irradiation of females, the rough estimate of risk under similar conditions is 0–900 cases per million births. The lower limit of this estimate assumes that the mutational sensitivity of the human immature oocytes will be similar to that of mouse immature oocytes whereas the upper estimate assumes that the human oocyte will respond in a manner similar to that of maturing mouse oocytes under conditions of chronic low-LET irradiation.

781. The risk from the induction of reciprocal translocations has now been estimated to lie in the region of about 30 to 1000 cases of affected individuals per million births per Gy of low-LET, low dose rate irradiation of males; for irradiation of females, the very indirectly estimated risks are lower (range of 0–300 cases of affected individuals per 10^6 births).

782. As in the 1977 report, the Committee has used a doubling dose of 1 Gy to estimate risks using the doubling dose method (the argument that the doubling dose is likely to be higher than 1 Gy was considered, but it was decided to keep the figure of 1 Gy for this Annex until more data on this aspect accumulates). The quantitative estimates of risk arrived at in this Annex are slightly different from those arrived at in the 1977 report. It is now estimated that under conditions of continuous irradiation at a rate of one Gy per generation (low-LET, low dose rate), the expected total increment in the frequency of genetic diseases is about 2000 cases per million births in the first generation (instead an estimated 6300 cases per million) and about 15 000 cases of affected individuals per million births at equilibrium (instead of 18 000 cases per million). The reasoning for this change has been: recent calculations indicate that for dominant and X-linked diseases, the first generation increment is 15% of that at equilibrium (thus lowering the number of cases from 2000 per million to 1000 per million); the conclusion of the Committee (arrived at on the basis of all available evidence) that the assumption of a doubling dose of 1 Gy for all chromosomal disorders (most of which are numerical anomalies of chromosomes) rests on particularly uncertain grounds; the Committee's current assessments relate only to the structural component of chromosomal disorders; the risk from the induction of numerical anomalies is considered to be very small.

783. In this Annex, the Committee has reviewed data that bear on severity or detriment associated with genetic diseases and has also made a first attempt to give some crude estimates of genetic detriment based on a number of assumptions, for spontaneously-arising and radiation-induced genetic diseases. Under the assumption that the average life expectancy at birth is 70 years (and thus, for a million liveborn, 70 10^6 years), it has been estimated that overall, spontaneously-arising genetic diseases cause about 2 300 000 years of impaired life per million livebirths and about 3 000 000 years of life loss per million livebirths. For a population exposed to low-LET low dose rate irradiation at a rate of one Gy per generation, the additional cases of genetic disease induced, would cause about 50 000 years of impaired life per million livebirths and an approximately equal amount of life loss per million livebirths in the first generation following the radiation exposure. At equilibrium, the comparable figures are, 340 000 years of impaired life per million livebirths and about 286 000 years of life loss per million livebirths. The Committee wishes to reiterate that these estimates are very crude ones, but are illustrative of at least one method to estimate genetic detriment.

VIII. SUGGESTIONS FOR FUTURE RESEARCH

784. In this Annex, the progress that has been made in mammalian and human genetics, cytogenetics, somatic cell genetics and in other areas pertinent to the evaluation of genetic radiation hazards in man has been reviewed, and revised estimates of genetic risks have been presented. The Committee feels that, in order to increase our precision in risk assessment, more research effort along the following lines will be useful (the order in which these are listed do not reflect the order of importance).

(a) Human studies
Continuation of surveys on hereditary diseases in human populations and correlation of clinical data and chromosomal defects; studies on the contribution of mutations to irregularly-inherited disorders; continuation of studies on genetic disorders such as ataxia telangiectasia in which the cells derived from patients suffering from the disorders show enhanced sensitivity to damage induced by radiation and by other mutagens, using all possible approaches and comparisons.

(b) Studies with mammals and other higher eukaryotes
Continuation of studies on the nature of radiation-induced dominant and recessive mutations at defined gene loci; studies on the induction of mutations in germ cells and somatic cells at low doses and low dose rates; studies on factors modifying radiation-induced genetic damage and on mutational assay systems in somatic cells; studies on the possible influence of genetic background on the induced frequency of dominant mutations in higher eukaryotes.

(c) Studies at the chromosomal level
Studies on the induction of reciprocal translocations (including primates and human testis material when possible) using cytogenetic techniques, especially at low dose rates and low doses of radiation; studies on the induction of structural aberrations in mammalian oocytes; studies on factors influencing the induction and recovery of chromosome aberrations in germ cells and somatic cells in suitable mammalian systems.

(d) Biochemical studies using suitable prokaryotic and eukaryotic systems
On the relationships between DNA damage, its repair and the origin of mutations and chromosome aberrations; mechanisms of constitutive and induced DNA repair by physical and chemical agents and their relevance for mutagenesis; mechanisms of regulation of DNA repair and of genetic recombination (possible role of hormones and growth factors) and their role in differentiation and carcinogenesis; DNA repair during gametogenesis; relationship of DNA lesions to changes in DNA sequences.

(e) Research on biological dosimeters to monitor radiation exposures
New approaches on the use of chromosomes as biological dosimeters; development of biochemical and immunological techniques for monitoring changes in DNA sequences and their application to estimate cumulative doses arising from exposure to physical and chemical agents.