B. GENETIC VARIABLES

1. Species

279. Few systematic comparative studies have been made on the influence of the species on the neoplastic response in mammals, and most of the data available have been obtained in the rodent. This problem has been discussed on the basis of the limited available information in previous reports of the Committee (669, 670) and also by various reviews (69, 672, 675, 678). It should be emphasized that the question of species susceptibility to radiation-induced neoplasia has far reaching implications, both theoretical and practical. In fact, it relates not only to the obvious need of extrapolating from one species to another, not only the carcinogenic effect, but also the relationships between latency time and life-span of the species (69). It is also relevant to the relationships between the spontaneous incidence of certain tumour types in certain species versus the radiation susceptibility of these animals to the same tumours.

280. Concerning tumours arising after external irradiation, it has often been pointed out (69, 670) that the variation between species appears to be considerable and that in any case, it seems larger than that observed after internal irradiation (456). Although it is true that virtually any type of tumour can be induced in any species by the appropriate doses and administration schedules (670), some species may develop certain tumours after rather low doses, or with relatively shorter latency times than others, so that the spectrum of malignancies found after irradiation in different species is extremely variable.

281. There are numerous examples of such differential effects. By comparison with other species, the mouse ovary is an example of an exceptionally high sensitivity to tumour induction. The rat is reputed to be considerably more susceptible to kidney tumour induction by 500 rad whole-body x irradiation than other species, in particular the mouse (36), but the apparent resistance of the mouse may be attributed to the particularly long latency of these tumours or to different pathogenetic mechanisms operating in the two species (422). Doses of 300-500 rad are definitely carcinogenic for many species, but the burro after single and total multiple doses of 320-545 rad develops an important dose-related shortening of life, without evidence of tumours (67). Lung cancer after 3000 or 4000 rad in the chest occurs in 43 per cent of rats but only in 2 per cent of hamsters (239), and this observation is confirmed by continuous irradiation experiments (734). Rats may develop leukemia after irradiation, but in a less consistent pattern than mice (683), and so do guinea-pigs (581); Chinese hamsters, on the other hand, appear to be strikingly resistant (450). Data on the incidence of myeloproliferative disorders in animals other than rodents have been reviewed by Nielsen (491).

282. When five different species of rodents were continuously exposed to $^{60}$Co radiation, more bone and, especially, lung tumours appeared in rats than in hamsters: other differences in inducibility of lung and oesophageal cancer were also shown to exist between various mouse strains (734). The differences between species found in this latter irradiation experiment appeared, on the whole, to be smaller than the differences in spontaneous tumours. This fact has been interpreted as evidence that radiation is so effectively carcinogenic as to override minor variations in susceptibility (734). Data are still insufficient to draw a comprehensive picture, and it may be concluded that the genetic character of the species is probably the main factor in these variations, to which other physiological and environmental components could add secondary, but by no means unimportant, contributions.

2. Strain

283. The genetic variation of the susceptibility to long-term effects of radiation has been explored, particularly in inbred strains of mice, and discussed, for example, by Grahn (226, 225) and Grahn et al. (227). Data on the spontaneous incidence of various forms of leukaemia in several strains of mice and on their susceptibility to radiation induction have been repeatedly mentioned in this annex (see particularly paragraphs 45 to 49) and will not be examined again. The conclusions drawn by Upton and Cosgrove (683) and Upton (678) are still applicable since, in general, strains having low spontaneous incidence of a particular disease are prone to develop an increased incidence after irradiation, while high-leukaemic strains are not as susceptible. The presence of a viral agent and its vertical transmission may be seen, for some forms of leukaemia, as a necessary condition for interspecies variations of the disease, although the condition is not sufficient since it may be modified to a large extent by the immunological, hormonal and ambient factors discussed in chapter II of this Annex.

284. The analysis of chronic-radiation lethality data from many different mouse strains suggests that all genotypes respond to radiation according to a single primary-injury parameter that can be expressed as life-span shortening. Strain or genotype specific sensitivities to tumour induction are of a secondary genetic nature and may be separated from the basic injury under special conditions of exposure (224). Daily irradiation experiments have indicated, for example, that life-span shortening at doses below 6 rad/day can be associated with specific neoplastic disease, but at higher dose rates a non-specific component of the damage is detectable (227). Such observations agree with the general view that at sufficiently high doses genetic differences tend to disappear (670). Information concerning sex differences in tumour induction by external irradiation, can be found in paragraphs 68 and 73.

3. Internal irradiation

285. Although it is commonly held that the neoplastic response of different animal species is more uniform after internal than after external irradiation (69, 456, 670), differences in the tumour susceptibility in the former case are also quite apparent. For internal exposures, in addition to the genetic factors discussed.
anatomical and physiological factors could modify the neoplastic response through modifications of the dose received by the relevant cells. For example, differences with species and age of the trabeculation pattern of bones are quite substantial (544, 18), and they influence the dose to the osteogenic or haemopoietic target cells in the case of bone-seeking radionuclides. Metabolic differences in the retention and excretion of radionuclides between different animals have also been shown, even for closely related chemical substances, and when operating over a long time they will result in appreciable differences of the accumulated dose in the target tissues (680). Thus, extrapolation to man of metabolic patterns and of the ensuing neoplastic effects only appears possible after careful consideration of several animal species with different life spans. Evaluation of the relative species sensitivity should therefore be made after appropriate corrections for all the factors mentioned above, and on the common basis of the estimated dose to the target cells.

286. Attempts to express sensitivity in terms of tumour induction have been made in the case of certain alpha-emitting radionuclides (433), and it has been concluded, for example, that the dog and the mouse are very similar in their response to $^{226}$Ra, but that man appears to be less susceptible by an order of magnitude. After appropriate corrections, mice and dogs seem also to be equally sensitive to the oncogenic effects of $^{90}$Sr (188) or $^{228}$Ra (219) and, on the basis of the lowest dose of $^{90}$Sr proven to be carcinogenic for bone, mice, rats and dogs, would also appear to be very similar in their susceptibilities (434), while the hamster would be more resistant (64). The cat, on the other hand, has been reported as particularly sensitive to $^{41}$Sr induction of osteosarcoma (489).

287. Systematic comparisons between species and strains are, however, rather few. In one of them, 70-day-old CF1 and CBA mice were given the same activity of $^{90}$Sr and examined every week radiographically for bone tumours. CF1 animals developed the first bone sarcoma at 98 days, and from then on tumour appearance followed a normal distribution with the mode at 27 weeks. The average time from the first scoring of tumours to death was 39 days. In CBA mice, on the other hand, tumours started to appear later and the peak incidence was at 35 weeks. The presence of inhibiting host factors, the higher spontaneous incidence of osteosarcoma in CF1 animals and the different rates of tumour growth in the two strains might account for these effects (185). More recently, four inbred strains of hamster treated with multiple intracraniial administrations of $^{210}$Po absorbed onto hematite particles were found to have very different tolerances to the treatment and induction times of the lung tumours. However, the final incidence of the tumours was very similar in the four strains, varying between 33 per cent and 50 per cent (395).

288. Species differences in the response of dogs, rabbits and rats to subcutaneous injections of $^{210}$Po were also reported (490). An important difference in susceptibility to osteosarcoma induction by $^{224}$Ra between male and female mice of the NMRI strain (284) has already been discussed (paras. 250-252); this observation appears to be in contradiction with what is found in humans (636). Chameaud et al. (98) have recently compared the susceptibility to lung tumour induction by radon and its daughters in man and in rat. They have shown that the tumour incidence plotted against the accumulated exposure in working level months (WLM) is similar in experimental studies with animals and in epidemiological surveys in man. They concluded that the rat could be a good model system for analyzing this type of neoplasia.

In conclusion, until further progress in the specification of anatomico-physiological characteristics of the various species is made, and until assessments of the dose to the relevant tissues may be carried out with better confidence, the scanty available data appear to be unsuitable for meaningful generalizations.

C. AGE EFFECTS

1. Post-natal irradiation

289. The relationship between age and susceptibility to the induction of tumours has been explored by irradiating animals of different post-natal ages or, in other cases, by comparing animals irradiated in utero or after birth. The two cases will be considered separately. It has long been known that life-span shortening in mice and rats after single acutely delivered doses of radiation is frequently associated with tumour induction, even at relatively low doses. Life-span shortening changes considerably with the age at irradiation: it is maximum in juvenile animals, then decreases with increasing age and can rise again in very old animals (383, 384, 385, 386, 387, 451, 349, 313, 130, 682). These variations have been attributed in part to the long latency in the expression of the life-span shortening effect (682) and in part to an intrinsic age-dependent change of the animals' susceptibility (313, 385).

290. Concerning specifically the induction of tumours, only fragmentary data on age-dependent differences in response are available. Mice of the RF strain given whole-body doses of 100-300 rad at several ages from birth to 180 days, showed that their susceptibility to granulocytic leukaemia is minimal shortly after birth, reaches a maximum at about 70 days of age and then slowly declines thereafter. This trend occurs in both sexes, although in the male peak incidence reaches 54 per cent after 300 rad of x-rays, while in the female the incidence after the same dose at the same age is only 26 per cent (690). Susceptibility to the induction of thymic lymphoma in the same strain is maximal shortly after birth and declines later in life with natural thymic involution (690). These observations are in good agreement with data on strain A mice, where maximal susceptibility to induction of lymphoid tumours after 1000 rad of x-rays (given in 12 days) occurs at the age of 1 month or earlier, with a sharp decrease at 2 months and later (319). They are also in agreement with data on the C57BL mouse given fractionated daily doses of 50 rad of x-rays for 12 consecutive days, where the increased incidence in early life is also associated with a shorter mean induction-time (320), and with data on SAS/4 animals, where leukaemia incidence tends to decrease with increasing age at exposure (386).

291. Sensitivity to the induction of ovarian tumours in RF females appears to be high (61 per cent after
300 rad) between 1 and 9 days of age and to decrease progressively up to 70 days (26 per cent for the same dose) (573). A very definite age variation is also found in SAS/4 mice where a dose of 650 rad of 15-MeV x rays produces about a 30 per cent incidence of ovarian tumours at 10-20 weeks of age, whereas in younger and older ages the incidence is smaller and may even be less than the 15-per-cent spontaneous incidence observed in the non-irradiated control mice (386). In another report, the spontaneous incidence of this neoplasia appeared to be different in three different mouse strains (IC, XLII and C3Hf/A), but a whole-body dose of 300 rad of x rays produced in all strains a higher tumour incidence in mice irradiated at an age of 4-6 weeks than in mice irradiated at an age of 4-6 months (566). In LAFI mice the induction of ovarian tumours was likewise lower after irradiation at 1 year than after irradiation at 10 weeks of age (130), but no differences with age, between 5 months and 2 years, were found in CAFI mice (349). In Wistar rats, the induction of ovarian tumours was significantly larger when x irradiation was performed at 13 days of age with a single dose of 270 rad (560).

295. When RF mice were irradiated in utero 1-3 days before birth with 300 rad of x rays, it was found that the incidence of granulocytic leukaemia was nil, but it increased substantially when irradiation was performed after birth to reach an incidence of 30-50 per cent at 70 days of age. Thymic lymphoma was similarly not induced by irradiation in utero but was readily induced after birth. Ovarian tumours were significantly less common in mice irradiated in utero than post-natally. There are no data at lower doses, but it could be assumed that 300 rad is too large a dose for effective induction of these tumours, because at doses of this magnitude the dose-effect relationship observed in the adult could be already in the declining portion (see paragraphs 151 to 154) (690). In a study carried out in RF mice irradiated at various pre- and post-natal ages, life-span shortening was found to be minimal in animals exposed pre-natally except for males exposed to 300 rad at 14½ days of gestation. where survival time was very short. Mice exposed pre-natally showed stunting of body growth, microcephaly and premature glomerulocarcinosis but no increases in incidence of leukaemia or solid tumours were noted (682). These data appear to be generally in agreement with data on CF1 mice with doses of 100 rad of x rays, where the evidence for any increment in neoplasia following embryonic or foetal irradiation was equivocal. There was, however, much evidence of reduced growth and permanent stunting (580). It may thus be concluded from all these data that the long-term effects of pre-natal irradiation relate more clearly to growth disturbances than to tumourogenesis.

296. From the available data in the rat (560), it would appear that animals irradiated in utero with a single dose of 270 rad of x rays had a considerably higher short-term mortality, and the survivors showed growth retardation, microcephaly and micro-opthalmia. Genital tumours in the female increased significantly only after post-natal irradiation, and their relatively low incidence in pre-natally irradiated rats was attributed to hypoplasia of the genital organs. Other data on Wistar rats, irradiated on the 18th day of gestation, have been reported by Piontovski and Kalashnikova (535). The beagle dog foetus responds to continuous irradiation in utero in a manner similar to that of the adult dog, but no effects on tumour induction were reported. At a dose rate of 17 rad/day or less of 60Co gamma rays, beagle bitches irradiated continuously from conception delivered apparently normal litters. At 5 rad/day, all female offspring were sterile, and at 10 rad/day, all male pups were sterile (512).

297. From the fragmentary data available, which do not cover specific tumour types in many different strains and species, it could be concluded that pre-natal irradiation affects the growth and differentiation of mammalian systems, rather than their malignant transformation. There is no evidence that irradiation in utero of mice and rats may be more carcinogenic than irradiation of young or adult animals.

2. Pre-natal irradiation

298. Data on the relative susceptibility to tumour induction in animals irradiated before or after birth are relatively scarce compared to the data on pre-natal irradiation in humans. Up-to-date lists of references on this subject have been published (562, 458).

3. Internal emitters

299. Age-related differences in the susceptibility to the induction of tumours by internal emitters have at times been reported. In the case of radiotrastium intoxica-
tion, the mouse (3-20 weeks) (701), the rat (6-60 weeks) (646) and the dog (new-born to adult) (188) were apparently more sensitive to bone sarcoma induction in youth than in old age. However, when appropriate dosimetric corrections to account for body size and higher retention in the young animals were made, these differences disappeared, showing that there is no major age variation in the intrinsic susceptibility of the cells at risk. Similarly, a larger susceptibility to bone tumours was noted after injection of $^{144}$Ce (but not after treatment with $^{239}$Pu) in weanling rats than in adults (417).

299. Comparisons of tumour induction between pre- and post-natal ages have been made by Finkel and her group using foetal and infant beagle dogs exposed to $^{89}$Sr. At the level of 1.33 μCi/kg in utero, $^{89}$Sr was not acutely lethal, nor did it produce haematologic disorders and bone sarcoma, however, extremely abnormal skeletal growth was found (186). In a later report (188) on $^{90}$Sr, some tumours were seen at higher levels of contamination. Differences in retention were held responsible for differences in tumour induction with respect to post-natal exposures, but it was concluded, on the whole, that pre-natal exposure was not more carcinogenic than post-natal exposures at comparable levels of dose. In the pig, exposure of the foetus to $^{90}$Sr ingested by the mother, plays an important role in the development of leukaemia (517). It has been reported that the administration of $^{32}$P to pregnant mice produces a significant increase of the incidence of leukaemia in female offspring (272). Finally, the age dependence of metabolism and effects of $^{239}$Pu has been studied in the rat by Sikov and Mahlum (626).

300. The relative sensitivity of the thyroid gland to the induction of tumours by $^{131}$I was investigated by Waibnder and Spoden (729) in mice exposed in utero or at the age of 3 months. They found 3 benign and 4 malignant neoplasms in 109 mice irradiated in utero to 7800 rad but no tumours at all after irradiation of 91 animals 3 months old to approximately similar doses. It was therefore concluded that the foetal thyroid gland is more susceptible than the adult thyroid. In analysing other evidence (160, 627), Pochin (537) concluded, however, that the numbers involved are probably too small for any conclusive statement about a higher sensitivity of the foetal thyroid.

D. TISSUES AT RISK

I. General

301. As pointed out in a publication (291) of the International Commission on Radiological Protection (ICRP), no general criteria are available at present to make reasonable predictions of the susceptibility of various tissues to tumour induction by radiation on the basis of known properties of the tissues themselves. Tumour susceptibility is therefore an empirical concept derived from the observed frequencies of cancer in different tissues or organs, following a given radiation dose. In man such a relative scale of sensitivity to tumour induction has been derived, with possible use in establishing dose limits to individual parts of the body (291). An empirical procedure is perfectly adequate for practical purposes such as setting dose limits, but fails to identify and explain biologically the reasons why some tissues or organs are more prone to neoplastic transformation than others, in a manner which is obviously quite independent of the number of cells they contain or of the renewal rate of these cells.

302. In considering this problem, it has to be kept in mind that although the natural occurrence of tumours is a rather common phenomenon in animal populations, the probability that a neoplastic clone would be induced in cells exposed to radiation is quite low. Induced neoplastic transformation may therefore be regarded as a rare phenomenon (70, 429, 459). There are general hypotheses compatible with this fact and also with the extreme variability of cell susceptibility to tumour induction; they have been discussed at some length in chapter II of this Annex. Other basic questions have not yet been discussed in detail, such as whether all the cells in any one tissue or organ, irrespective of their state of differentiation, are susceptible to the induction of neoplasia or only some of them can be transformed, or whether it is possible to identify the cells in a population having a higher risk of neoplastic transformation by radiation. These questions have already been considered in the 1972 report of the Committee (670) with special regard to bone and skin tumours.

303. In a recent publication (430), Maynard and Clarke have discussed the relevance and importance of the number of cells at risk in studies of radiation carcinogenesis and have developed some biomathematical concepts to relate the transformation of these cells to radiation dose under various conditions of irradiation. Although the authors themselves recognize the limitations of such an approach in view of the complex reality of the biological mechanisms, the formulations proposed and the general consequences to be drawn provide a stimulus for a better definition of the relevant problems. In the following paragraphs the question of the cells at risk will be considered systematically in the light of available information, in an attempt to elucidate the problem of the differential susceptibility of cells to tumour induction and also to provide a rational basis for possible extrapolation of data between species.

304. Leukaemia. The comparative aspects of radiation-induced leukaemia in animals have been considered by Upton (678) and Kaplan (328). It is quite apparent that the histological type, the time of onset and the organ distribution of the reticular tissue disorders developing in the experimental animals are extremely variable. In the mouse, which is the species most extensively studied, the inducibility of leukaemia by external or internal irradiation and its type depend primarily on the strain and then on sex, age at irradiation, environmental factors, radiation dose and type of fractionation. All these factors have been considered at some length in this Annex. In this species the tumours of the reticular tissue may frequently take the form of a lymphoma (in the C57BL strain typically) which appears to arise early in life in the thymus and then to spread in some cases to the thoracic lymph nodes or to other lymphatic and haemopoietic districts (326). Alternatively (and more
rarely) a myelogenous granulocytic leukaemia may occur, particularly in the RF strain, involving primarily the marrow, spleen and liver and producing peripheral blood leucocytosis (685).

305. In the mouse another form of non-thymic reticular tumour of complex and still uncertain classification has also been observed, which generally arises late in life, involving primarily the reticular cells of the spleen and of the abdominal lymph node and which may then extend to other organs where reticular tissue is present (683). In addition to anatomico-pathological and epidemiological evidence, there are also radiobiological reasons to keep these tumours separate from the others since radiation appears to depress, rather than enhance, the incidence of the diseases (see paragraphs 117 and 119). In the rat the development of radiation-induced leukaemia is less well documented as to form and induction time and rate, but myeloid leukaemia, leukaemia and reticular diseases have been reported (683, 161). In guinea-pigs, leukaemias of a chronic lymphatic type (683) or lymphosarcomas (581) have been described. Swine irradiated chronically with $^{90}$Sr develop high incidences of myeloproliferative disorders, including myeloid, lymphatic and stem cell leukaemias (278), while beagle dogs submitted to chronic irradiation by external sources (205) or by $^{90}$Sr feeding (166) show highly proliferative infiltrating forms of myeloid leukaemia.

306. In view of the diversity of these syndromes and in the absence of any indication as to whether and to what extent any of them might be akin to the human leukaemias, attempts to identify the nature and the number of the cells at risk on the basis of our present knowledge may only be regarded as an "academic pursuit" (459). For all practical purposes, in fact, reliance must be placed mainly on the few observations made in the cases of human leukaemia (562). The virus-induced thymic lymphoma of the mouse, the complex pathogenesis of which has been sufficiently well documented, may be a good example of the difficulties involved in assessing the cells at risk.

307. As already discussed in paragraphs 46 to 48, anatomico-pathological observations and the effects of thymectomy and thymus reimplantation have shown that thymus is the target organ for the action of the virus. Further experimental evidence has identified the immature lymphoid cells of the thymus as the target cells in this tumour system. However, irradiation of the target organ is not sufficient, as such, to induce lymphoma without irradiation of the haemopoietic system and, in addition, other micro-environmental, humoral, immunological and constitutional factors are essential requirements for the expression of the disease. The relative abundance and the susceptibility of the target cells to the virus are very dependent on their state of differentiation and on the age and constitution of the animal (326, 327). It is clear, however, that since a viral agent is present at the origin of the disease, the problem is primarily one of investigating quantitatively in vivo the interactions between target cells and virus, since radiation acts primarily through a change in the host-virus relationships. The evidence on this point has been reviewed by Kaplan (327).
In vivo systems and be useful not only for investigations of mechanisms but also for practical applications (564). Suitable in vivo models ought to be developed to fill the gap. These models should have under better control the number and the functional characteristics of the cells that appear to be the likely candidates for neoplastic transformation.

312. Ovary. Irradiation of the ovary leads in general to a precocious reduction of the number of oocytes and ovarian follicles (14). With the exception of these cells, practically all the other cells forming the organ (lutein and granulosa cells. mesothelial and endothelial cells) appear susceptible to neoplastic transformation by radiation (675). Ovarian tumours are particularly frequent in the mouse, where doses as low as 50-100 rad of low-LET radiation may produce peak incidences of tumours (107). They appear to be responsive to hormonal mediation by the pituitary gonadotropins or by the controlateral non-irradiated ovary, in the case of partial-body irradiation (211). No special cell may be identified as being characteristically at risk in the case of ovarian carcinogenesis.

313. Lung. The pulmonary tissue is very complex, both morphologically (633) and functionally (623). A considerable spectrum of tumour types occur spontaneously (492) or are induced in domestic animals after pulmonary deposition of radionuclides. Bronchio-alveolar carcinomas have been reported in dogs (111), squamous cell carcinoma, adeno-carcinoma and hemangiosarcoma in the rat (358, 741) and fibrosarcoma. squamous cell carcinoma and bronchiolar carcinoma in the mouse (657). It is apparent, therefore, that all cells of endodermal origin composing the lung may give rise to neoplasia. With succeeding degrees of anaplasia, the cells may express any of the characteristics of the embryonic pulmonary endoderm (633). Connective and vascular tissues are also capable of neoplastic transformation. The role of non-specific fibrosis of the lung, preceding or accompanying the lung neoplasia has been discussed (111) and it has been concluded that it may be an important contributing factor when a specific carcinogen is present. Bronchiolar and alveolar proliferation into scarred areas of the lung gives rise to proliferative and metaplastic cells, which are often the primary changes of the lung in the case of inhaled compounds (490). Pulmonary adenomas are frequently found in mice both spontaneously and following whole-body and partial-body irradiation (see paragraphs 119 and 120). They are frequently of alveolar origin, with a variety of histological patterns (641, 642, 65).

314. Mammary gland. Most of the studies on the mammary gland are confined to breast tumours of the Sprague-Dawley rat. In this system, irradiation of other tissues except the target tissue is not required to elicit the neoplastic effect (52, 616, 617, 606). The intact function of the ovary is however required for maximum expression of the neoplastic effect (138). By histological and biological criteria, the tumours produced are described as mammary adeno-carcinomas, adeno-fibromas or fibroadenomas, according to the relative abundance of fibrous and adenomatous tissue (611). No information is available on which are the possible cells at risk, although this system, which has good characteristics for oncological studies (614), would appear to be open to quantitative developments on a cellular basis. It is of interest to note that very recently an experimental technique to quantify radiation effects on rat mammary tissue has indeed been developed by Clifton et al. (112).

315. Kidney. In the Sprague-Dawley and the FAF1 rat, radiation-induced kidney tumours appear to be tubular in origin, while in mice they seem to arise from the glomerular capsule with secondary tubule involvement. Nephrosclerosis and arteriosclerotic changes would appear to play a major role in their pathogenesis (36). In a thorough study of the histogenesis of kidney tumours in rodents (422). Maldague has confirmed that in the absence of radiolesions, leading to renal atrophy and nephrosclerosis, no tumours are found in Wistar rats and in mice of the strain XVIII. Concerning the tumour types found in the rat, cortical adenomas originating from cells of the convoluted tubules, carcinomas with various degrees of anaplasia and a few sarcomas were described. In animals locally irradiated in the kidney and then uninephrectomized controlaterally, it was found that focal localized proliferation of the epithelial cells at the junction between cortex and medulla (which are probably the induced cells transformed to malignancy) give rise very frequently to the neoplastic nodules. Cortical adenoma, cortical carcinoma and transitional cell carcinoma were also described in rats (569). In the mouse, tubular adenomas and clear cell carcinomas, similar to the tumours found in rats, were also seen (422). It may therefore be concluded that the available evidence points particularly to the tubular cells as the cells susceptible to neoplastic transformation, but that tumours arise in other degenerative and sclerotic lesions. The role of these pathological components is still unclear.

316. Skin. The histopathogenesis of skin tumours after irradiation with low-energy beta particles of the CBA/H mouse has been described by Hulse (421). Epidermal (papilloma and squamous carcinomas), dermal (mainly fibrosarcomas, but also fibromas and hemangioendotheliomas) and subdermal (fibrosarcomas) tumours were found. In spite of the fact that early and late effects on skin and skin annexes were carefully followed and described, it was not possible to find any correlation between the degree of non-neoplastic skin damage (depigmentation, ulceration, scars) and the likelihood of tumour development. It was definitely stated however that, contrary to other reported results, tumours seemed to arise as a direct effect on irradiated cells and in areas of the skin which did not appear to have suffered gross radiation damage. In a later report on dose fractionation, the lack of correlation between skin damage and tumour formation was confirmed and the relative independence of skin tumour production on the mode of exposure to radiation suggested, in the opinion of the authors, the existence of a mechanism of tumour induction of a permanent and cumulative type, such as a somatic mutation (287). It was further argued that the sensitivity of the transformed cells to radiation killing might be exceptionally low compared to the values commonly found in mammalian radiation biology (288). Other results in the mouse are particularly interesting for a discussion of the cells at risk (369). By skin irradiation with helium ions of different energies it was found that
the number of tumours increased with increasing penetration of the beam, in agreement with other data relating to the rat to be discussed below. The dependence of tumour formation on acute skin damage was in this case partially confirmed.

317. The situation in respect to skin tumour induction in the rat appears to be quite different, in the sense that in the skin of this rodent there is an association between hair follicle damage and the magnitude of tumour response. The incidence of atrophic follicles reaches a peak at about the same dose at which maximum tumour yield is found, and also there is good correspondence between the shape of the dose-effect relationships for the two types of damage (6). In a study performed with electrons of several energies it was established that, when the dose delivered with all energies is normalized at a depth of 0.27 mm, the dose-incidence curves coincide, indicating that the cells located at this depth from the surface (identified as the cells at the bottom of the resting hair follicle) are to be considered as the cells at risk for this type of tumour in the rat. In recent experiments (7), a comparison of the skin tumour response of mice and rats showed that the markedly lower susceptibility of the mouse was due to the failure to develop adenocarcinomas, which are the predominant type of neoplasia in the rat. Marked differences in hair follicle injury in the two species were noted. In particular, the mouse develops relatively little follicular atrophy, and hair follicles appear to be either intact or destroyed: in addition, the mouse hair follicle appears to be more sensitive by a factor of about 2. The susceptibility to the induction of connective-tissue tumours, on the contrary, is roughly similar in the two species.

318. Bone. Detailed descriptions of the histopathological changes observed in bone after irradiation by bone-seeking nuclides have been provided by Nilsson (499, 509, 647, 494) and Vaughan (703). It is doubtful, however (459), whether the described microscopic damages have any relation with tumour induction since the occurrence of tumours has been shown to be the most sensitive parameter and indicator of radiation damage to the bone (185, 703). Histopathological evidence points to the following cells as the most important cells at risk in the case of internal irradiation by bone-seeking nuclides: the osteogenic tissues of the bone surfaces giving rise to osteogenic and chordogenic sarcomas; the bone marrow for all haemopoietic, lymphopoietic and myelopoietic disturbances, and the epithelial cells in close contact with the bone, giving rise to epithelial tumours of the mucous membranes of the head. The case of the marrow has already been discussed in connection with leukaemia (paras. 304 to 311), and it should be pointed out that in rare cases other cells than the haemopoietic elements might be susceptible to neoplastic transformation, as for example in the case of haemangial tumours.

319. Concerning osteogenic tissue tumours, chromosome-marker experiments on chimerized CBA mice have definitely proven that osteosarcomas developing after $^{90}$Sr treatment derive from the osteoprogenitive tissues of bone and not from the pluripotent cells of marrow (26. 48). The cells at risk are therefore to be found among the progenitor cells of the bone-forming tissue, namely the pre-osteoblasts and pre-osteoclasts. These cells lie within 10 μm of the endosteal and periosteal surfaces (703) and therefore are at risk in cases of irradiation by both alpha and beta emitters of all energies deposited in bone. These cells are the ones becoming malignant due to an abnormal proliferation following irradiation while maintaining the functional activity of the original progenitors (499. 4). The particular location of these cells explains the observation that bone tumours are more frequently of endosteal than of periosteal origin, on account of the fact that endosteal surfaces are far larger than periosteal. It also accounts for the observation that surface seekers are more effective than volume seekers for bone tumour induction, since the dose delivered to the target cells is higher in the first case.

2. Conclusions

320. It may be concluded that further refinements of our knowledge about cells at risk for neoplastic transformation are of extreme importance, both for elucidation of mechanisms and for better systematization of data. The problem has been tackled so far (and in some cases qualitatively solved) only in those tumour systems where indirect mechanisms of induction and promotion are relatively less important, and for which the direct action of radiation on the tissue at risk appears to be the determining factor for tumour induction. A more quantitative approach seems now required, as well as the development of investigation techniques aimed at the establishment of links between observations in vivo and model systems of cell transformation in vitro.

VI. SUMMARY AND CONCLUSIONS

A. GENERAL

321. A selective review of experimental radiation carcinogenesis has been carried out in order to set the old information and the newest acquisitions in the more general framework of cancer induction as a biological phenomenon, and with the ultimate aim of identifying mechanisms or regularities which might facilitate the interpretation of human data. The reviewed information is both qualitatively and quantitatively inadequate for numerical prediction of tumour induction rates in man, even though projections between species may be considered as the main objective of animal experimentation. The experimental data are, however, invaluable for understanding mechanisms, for the formulation of useful generalizations applicable to all species, and for the development of models to test specific hypotheses of tumour induction.

B. METHODOLOGY

322. Several methodological approaches have been followed in the past, ranging from the large-scale epidemiological-actuarial analysis to the morphological
and pathological approach, and from biochemical and molecular experimentation to the more recent analysis of model systems of carcinogenesis. All of these have advantages for specific purposes, but each of them appears inadequate for comprehensive solutions of general validity. The long-term nature of the work in vivo is such that it requires a careful choice of experimental animals, high standards of animal husbandry and maintenance, advance planning of the size of the experiment, selection of appropriate biological end-points, quantitative observations on the relation of dose and time parameters with the carcinogenic effect, and careful statistical evaluation of the age-specific tumour induction rates. Although continuous improvements on these points are evident in the work reviewed, much could still be done in order to refine the technical requirements for reliable investigations and to standardize the working conditions on an international basis to permit a wider applicability of the individual observations.

C. MECHANISMS

323. The probability of radiation-induced changes in individual cells leading to the appearance of tumours is very low. Neoplastic transformation is presently visualized as the end-result of a complex chain of etiological and pathogenetic events that confer upon the tumorous cell an irreversible and unlimited capacity for proliferation outside the normal control mechanisms. Unlike some chemical carcinogens, radiation can both initiate and promote neoplasia; it may also interact with other physical, chemical or biological carcinogenic agents with variable results under different circumstances.

324. The genetic foundation is common to the currently accepted hypotheses of radiation carcinogenesis, and the recent advances in molecular genetics and virology are gradually closing the gap between the classical "theories" of the "somatic mutation" and "viral" induction of cancer. Vertically transmitted RNA oncoviruses have definitely been shown to be at the origin of some radiation-induced murine tumours, and in the very few cases where the analysis of mechanisms has been carried to a sufficient depth radiation has been found to act through a modification of the host-virus relationships, in a complex interplay of physical, genetic, micro-environmental, hormonal and immunological actions. Attempts to study cell transformation by radiation on cultured cells have been initiated and should be pursued to investigate the mechanisms of cancer induction in the absence of other interactions operating at the whole-body level.

325. There are data to show that a transient non-specific immunosuppression caused by fairly high doses of radiation may have a promoting role in the development of radiation-induced tumours. Moreover, the viral infection per se, acting through very specific cellular mechanisms, can cause a depression of the immune reaction in cases where tumour viruses are implicated. Although these effects would only appear to have a secondary role, the questions as to their actual importance and their relevance at low doses and dose rates remain unsolved. Similarly, no definite answer can be given about the role and the mechanisms through which hormones could alter the susceptibility of certain radiation-induced tumour systems in vivo, although the animal's hormonal balance may affect the carcinogenic response of some systems. Cellular proliferation is implicit in the notion of a promoting action and is certainly required for tumour progression. Cell division is however a non-specific type of tissue reaction to radiation-induced cell depletion, and it is very difficult to attribute to such an action a primary or a secondary role in tumour induction. Other environmental conditions concerning the host microflora, physical conditions, chemical substances and biological treatments may affect to various extent the neoplastic response to radiation.

326. In conclusion, a variety of biological mechanisms through which radiation can induce tumours have been identified and, in some cases, analysed. The evidence shows that mechanisms that appear very important for a particular tumour system may, under a given set of experimental conditions, be less important or even not relevant for the induction of other neoplasms. Our present knowledge of mechanisms therefore provides partial answers, but it is still insufficient for conclusions of general validity.

D. DOSE, DOSE RATE AND RBE

327. Among the physical parameters affecting tumour induction, the dose, dose rate and quality of radiation have been considered. Concerning the dose, three major patterns of tumour response can be identified. The first refers to those tumours which do not seem to be effectively induced in the usual range of low to mid lethal doses but may however be induced at higher doses. The second refers to those tumours for which the dose-effect relationship has a negative trend. The third, and probably the most common pattern, is more complex, showing an initial rise at increasing doses, a peak or a plateau at some intermediate level and, in many cases, a final decline of incidence at sufficiently high doses.

328. The peculiarities of each tumour-model system which have been pointed out repeatedly in this Annex are such as to prevent undue generalizations, particularly when they might apply to all systems. There are also difficulties in interpreting tumour induction curves in the animal on the basis of simple mechanisms of action, in view of the complex interplay of primary and secondary factors, also discussed at length in this Annex. With few exceptions, the existing data come from observations at doses above 50 rad, since lower doses have generally failed to cause an increased tumour incidence large enough to be quantified in experiments of the usual size involving no more than 100 animals per dose-group. Even in those instances where the incidence of a particular neoplasm has been observed to increase with dose, the data usually do not suffice to define the dose-effect relationship unambiguously in the low-to intermediate-dose region.

329. With one exception, namely the rat mammary tumour. the dose-incidence curves obtained at high dose rates with low-LET radiation generally increase in slope
with increasing dose in the range from 50 to a few hundred rad. The data are not incompatible therefore with the notion that an appreciable dose-squared component might be present in these curves, which would imply that linear interpolation or extrapolation from the observed data would tend to overestimate the risks to be expected at low doses (less than 5 rad) and at low dose rates (less than $10^{-3}$ rad/min). With high-LET radiation, on the other hand, the dose-effect relationships are more often compatible with a linear non-threshold response, and their slopes vary relatively little with dose and dose rate. Those instances where the tumour induction curves are seen to decrease in slope at high doses and dose rates may be interpreted to result from two independent dose-related phenomena: neoplastic transformation of the susceptible cells, the probability of which increases with increasing dose, and survival of the transformed cells, the probability of which decreases as a function of dose.

330. Detailed analyses of the dependence of the RBE of different ionizing radiations on dose and dose rate are only possible at present for very few experimental tumours. It appears that the efficiency of high-LET radiations for tumour induction is higher than that of low-LET radiations, and the efficiency often changes at different dose ranges. At doses of 100 rad or more the RBE is often about 1, and it shows a rather general tendency to increase at low doses and dose rates, owing probably to the form of the dose-induction relationships, where the linear component prevails in the case of densely ionizing particles, while the quadratic component might prevail, at least at high doses and dose rates, in the case of low-LET radiation. A noteworthy example of this type of effect is seen in the acceleration of breast-tumour development of the Sprague-Dawley female rat, where effects of fast neutrons are detectable at doses of 0.1 rad, with corresponding high values of the RBE.

331. A spreading in time of the delivered dose through a decrease in the rate or through fractionation of the dose results in general in a decrease of the oncogenic effect of radiation, following some inverse function of the exposure time. It is however difficult to assign precise values to this sparing effect, since the shape of the dose-induction relationships is often altered by the change of dose rate. The magnitude of the reduction of carcinogenic effect at low dose rates is often found to be smaller with high-LET radiation. Departures from this general pattern have been documented in some tumour systems but are attributed to the pathogenetic mechanism of the particular system and not regarded as exceptions to some fundamental radiobiological mechanisms. Recovery processes are responsible for the sparing effect of fractionation and exposure rate: their nature and kinetics in the case of neoplastic induction remain largely unknown.

E. INTERNAL EMITTERS

332. Carcinogenesis by internal emitters has been considered as a special case since, in addition to the general problems of radiation carcinogenesis, it poses other problems related to the inhomogeneity of the dose distribution in time, and also to the concentration of the radionuclides in some organs or tissues. In addition, the comparison of effects of various nuclides is often made uncertain by the difficulties of assessing the dose to the target tissues, because of the peculiar behaviour of nuclides having different physical and chemical characteristics and, therefore, different rates of uptake, retention and tissue localization. The cases considered in detail cover the induction of bone tumours and myeloproliferative diseases by beta- and alpha-emitting bone-seeking radionuclides and the induction of lung tumours following inhalation of alpha emitters in various animal species where data are available. A discussion of the probable form of the dose-induction relationship for the various types of neoplasms has also been included.

333. When the special factors mentioned above are taken into proper account, it appears that the qualitative picture emerging from these data is compatible with the concepts developed from the analysis of the effects of external radiation. The general form of the dose-induction relationships, the fact that at low doses beta emitters tend to produce curvilinear responses and alpha emitters linear ones, the higher efficiency of the alpha emitters, and the sparing effect of low dose rates of sparsely ionizing radiations, are all phenomena that can be documented in the case of internal irradiation. This strongly suggests that, with appropriate corrections, the general knowledge on tumour induction may safely be applied to both irradiation conditions.

F. BIOLOGICAL VARIABLES

334. Among the biological variables influencing radiation carcinogenesis, the effects of species, strain, sex and age at time of irradiation have been reviewed. Even with the lack of systematic data, it is possible to conclude that the genetic character of the species and, within species, of a particular strain, represents a major determinant of the carcinogenic response, to which other physical, physiological and environmental parameters may contribute as secondary variables. Genetic differences in susceptibility to tumour induction by radiation are especially manifest at low doses: with increasing doses and dose rates they tend to be cancelled. There are also quite definite changes in the susceptibility to the induction of various types of tumours as a function of the post-natal age at which animals are irradiated. Irradiation of experimental animals in utero would not appear, on the whole, to be more carcinogenic than irradiation during post-natal life.

335. Finally, the existing data on the problem of the tissue and cells at risk for the induction of various tumours have been reviewed. It appears that this subject may have important implications for the elucidation of mechanisms and for possible practical applications. Existing data relate, however, only to those tumour systems where the direct action of radiation on target cells appears to be the determining factor for tumour induction. A better quantitative definition of these problems by development of more sophisticated techniques of analysis at the cellular level is desirable.
VII. NEEDS FOR FURTHER RESEARCH

336. The previous report of the Committee (670), as well as other publications (563, 291, 564, 419), have indicated some topics of special interest for future research. Adequate selection of research topics is particularly necessary in carcinogenesis studies because of their long-term nature and of the major organizational and financial efforts they require. The Committee calls attention to the role of the experiments on animals for the eventual elucidation of the mechanisms involved and therefore for the extrapolation of data to man. Considerably more data would be required to extrapolate the few available data in humans to different conditions of exposure. A systematic analysis of the effects of the main radiobiological variables in experimental systems could make possible the refinement of the risk estimates in man by a considerable factor within the next decade (564).

337. Concerning the strategy of future experiments, special emphasis should be placed on the development and analysis of models of the carcinogenic action, rather than on large-scale experiments. It seems likely that some elucidation of the many factors interacting in carcinogenesis could be obtained through the study in depth of conceptually simplified experiments. A multidisciplinary effort of a quantitative, rather than a descriptive, nature seems to be called for. For example, our present knowledge of some tumour systems in vivo makes it possible to select as an indicator the induction of tumours in a definite cell line at risk, rather than the gross appearance of any type of tumours in a population of animals. The use of more relevant end-points may provide a rational basis for possible extrapolations from one species to another.

338. It is generally recognized that the area of greatest applied interest is, and will continue to be, that of the effects of low doses and dose rates. It follows that the experimental efforts should be concentrated in that area. It should also be emphasized that experiments on tumour induction as a function of dose and dose rate in selected animal model systems appear at present to be the type of studies that might best contribute to further advancement. Careful planning and design of the experiments, refined statistical analysis of the data, and also a better standardization of the techniques used in different laboratories would strengthen the validity of the observations.

339. The following are some specialized topics requiring further elucidation, and for which currently available techniques would justify increased efforts with reasonable hope of progress. The topics are given in the same order as they have been discussed in the main text of the report, without any indication of priority.

(a) The somatic mutation hypothesis of tumour induction and its possible relations with the viral hypothesis could be investigated by means of studies of the damage and repair to DNA and genetic structures in transformed cells following irradiation or viral infection. An attempt should be made to clarify the relationships between neoplastic transformation of the cell and its capacity for further reproduction and other possible damage to important cell structures.

(b) On the basis of the data reviewed, it seems that different radiation-induced tumour systems do not have the same etiopathogenetic mechanisms: on the contrary, it seems that the relative importance of each mechanism is different in different systems. By testing this relative importance, better elucidation of the role and relevance of the various inductive and promotive factors in different experimental situations could reasonably be expected. Further experiments of the role of various co-carcinogenic factors with particular relevance to the presence of potentiating effects in regard to tumour induction would also be desirable.

(c) Since viral agents are implicated in the induction of at least some radiation-induced tumours, attempts to demonstrate the viral origin of other tumours should continue together with studies on virus activation and on the immunological mechanisms interacting in such cases.

(d) The development of models of cell transformation in vitro should actively be pursued to clarify the kinetics of cell transformation and its relationship to cell inactivation. Several possible models have already been identified (564), and it is not unreasonable to expect that some of the classical radiation-induced tumour systems in vivo could also be subjected to more quantitative in vitro analysis.

(e) For in vivo studies, the identification of the cells at risk would greatly help in identifying the mechanisms and unifying the observations. Quantitative analysis of the capacity for division and differentiation of these cells, in relation to the transformation events, would provide important links between in vivo and in vitro studies. Furthermore, the period elapsing between the initial transformation of the cell and the clinical manifestation of the tumour should be studied together with the influence of immunological, hormonal and other promotive factors.

(f) More experimental data are required to establish with precision the dose-effect relationship below 10 rad for many tumour systems. Studies of the relative importance of the linear versus quadratic components in dose-induction curves would be very valuable in this context. Better knowledge of the nature of such relationships would considerably improve the confidence in the estimation of the risks of tumour induction in man at low doses and dose rates (see Annex G). The development of model systems in vitro and the analysis of the data by means of refined theoretical models could help identify the relevant trends. It should be pointed out, however, that any conclusion will have to be confirmed by data in vivo, which are extremely difficult to obtain, particularly in the less susceptible species.

(g) The mechanisms involved in the determination of the nature of the dose-effect relationships at medium to high doses are uncertain. It seems reasonable to assume that the identification of such mechanisms might indirectly help establish the possible shape at very low doses.

(h) There are few studies of RBE for tumour induction. More effort is needed in this direction, particularly with the most sensitive systems which could allow an assessment of the RBE at low doses and dose rates, for which information is definitely insufficient.
(i) The lower efficiency with which irradiation at low dose rates induces tumours, which has been qualitatively described for some systems in vitro, should now be explored in other tumour systems and evaluated quantitatively.

(ii) Although it seems reasonable to postulate that repair phenomena with various time constants are operating at various levels of the biological organization, the nature and kinetics of such repair mechanisms in the case of low dose-rate irradiation are still essentially unknown and require elucidation.

(k) Further comparative studies of tumour induction in different species and strains would increase our confidence in interspecies extrapolations. In this respect it would be desirable to test in other animals the conclusions reached in rodent experiments about the effect of the main physical and biological variables. However, no large-scale studies should be undertaken without sufficient scientific justification, which could be based on the relevance of the information to be gained for human assessments.

(l) The importance of systematic studies of tumour induction in various animal species and in animals of different size and age should be recognized. These studies should attempt to correlate the induction of neoplasia with the number and functional state of the target cells at risk of malignant transformation in any particular tissue.

(m) The conclusion that irradiation in utero is not more carcinogenic than in the young or in the adult animal appears to be based on rather scanty data. A more systematic search covering various tumour types in different strains and species and relating tumour induction with the rate of maturation of the relevant organs would considerably strengthen that preliminary conclusion.
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