ANNEX I

Genetic effects of radiation

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1. The 1977 UNSCEAR report [U1] presented a detailed review of the genetic effects of ionizing radiation. This Annex is aimed at updating the 1977 report, especially those parts that require significant revisions in the light of new data. Particular emphasis is given to those data that are relevant to the evaluation of genetic radiation hazards in man.
I. HUMAN DATA

A. NATURALLY-OCcurring HEREDITARY DISEASES AND DEFECTS

2. In the 1977 report, the results of the British Columbia Survey [T1] on the frequency of liveborn individuals affected by hereditary or partially hereditary defects and diseases were presented. These results and considerations based on the degree of completeness of reporting in the Registries used as data-source and possible biases due to delay in disease onset, migration etc., allowed an estimate of 9.44% for the total frequency of diseases believed to be of genetic origin (0.12% autosomal dominant and sex-linked diseases, 0.11% recessive and 0.20% chromosomal ones, 4.28% congenital malformations and 4.73% other multifactorial ones).

3. The Committee reappraised the above figures, taking into account Stevenson's data from the Northern Ireland Survey [S1], the results from several ad hoc surveys for specific dominant conditions (reviewed in reference [V1]), data from newborn surveys for chromosomal anomalies and the uncertainties involved in the aetiology of diseases classified as congenital malformations, multifactorial diseases and irregularly inherited diseases. It was concluded that for the purpose of estimating genetic radiation hazards in man, it is appropriate to use the following revised figures (adding up to a total of 10.5%): (a) 1.6% dominant and X-linked diseases; (b) 0.1% recessive diseases (excluding those maintained through heterozygous advantage); (c) 0.4% chromosomal diseases; and (d) 4.3% congenital malformations, and 4.7% other multifactorial and irregularly inherited diseases, together, 9%.  

4. Some new information has become available since the 1977 report. Czeizel [C1] has published the results of a nation-wide survey on congenital malformations in 1970 in Hungary. The classification of these malformations into the various categories and sub-categories has been based on the International Classification of Diseases [W1], with some modifications. The data cover a 7-year period (1970–1976) and involve a total of 1,188,509 births of which 10,658 were stillbirths, the rest being livebirths. The frequency of malformed babies varied from 2.2% in 1970 to 3.7% in 1976 with an average of 3.1%. These figures are very similar to that given as a minimal estimate (3.6%) for the British Columbia data by Trumble and Doughty [T1]; the latter estimate however relates to livebirths only. Czeizel et al. [C2] noted that, for the period 1972–1975, the incidence of multiply-malformed babies ranged from 7.5 to 8.5% of all notified congenitally malformed ones suggesting that from 2.6 to 2.9 per 1,000 total births showed multiple malformations; the majority of these were found to be severe: 6.9% of such babies were stillborn and 45% of those that were liveborn died during infancy.

5. The Hungarian Congenital Malformation Monitor, operational since the beginning of 1977, takes up of the so-called indicator congenital malformations1 to monitor the temporal and spatial trends in the incidence of these malformations. During the period 1973–1976, trends for a continuous increase were found for three of the indicator traits: congenital dislocation of the hip, congenital limb reduction and hypoplasias [C3]. Czeizel [C3] suggests that these trends may be related to the more complete notifications although the increase in limb reduction deformities is only partly explained by this factor. Other trends noted were of a transitional type, i.e., increases for short periods (e.g., anencephaly in the fourth quarter of 1974; spina bifida in the second quarter of 1974, in the third quarter of 1975 and in the fourth quarter of 1976; cleft lip ± palate in the third quarter of 1973). While statistical analysis revealed that these trends are not due to chance, Czeizel has stressed the point that the contribution of three possible technical biases (changes in diagnosis, notification and evaluation of the given congenital malformation) must first be excluded before the trends become amenable to meaningful interpretations or to the search for causal factors.

6. In their extensive paper, Myrianthopoulos and Chung [M52] have reported the results of a comprehensive prospective study of congenital malformations in children born in the Collaborative Perinatal Project (CPP: a cooperative endeavour of twelve institutions throughout the United States and the National Institute of Neurological Diseases and Stroke of the National Institutes of Health, set up in 1959). Pregnant women have been followed from the first months of their pregnancy through labour and delivery and the children born to "project mothers" were followed up to one year of age. The project population was classified by the researchers in the United States as about 45% "white" and 55% "non-white".2 The collection of information, medical examinations and laboratory tests have been done in uniform fashion and according to pre-established protocols.

7. The total data pertain to 53,394 single deliveries including 52,390 live births and 1,004 foetal deaths; in 137 of the total, the sex of the children was not known either because they were miscarried foetuses or because, for one reason or another, they were lost to the study. All analyses were based on 53,257 deliveries with known sex; 15.6% of the children (8,288 out of 53,257) were born with known malformations. 13.0% or 6,906 with single and 2.6% or 1,382 with multiple malformations.

8. The authors point out that the high rate of malformations found in this study (relative to others published in the literature) can be explained on the basis of the period of time through which the malformations were observed and the conditions which made almost complete ascertainment possible: only about one-third of malformations observed during the first year of life were diagnosed at birth.

9. Other interesting findings that emerged from this study include the following: (i) major malformations (e.g., anencephaly, microcephaly, congenital dislocation of the hip, cataract, cleft palate, cleft lip, pyloric stenosis, hypoplasia, cystic kidney etc.) were present in about 7% of the children, minor malformations (e.g., polydactyly, syndactyly, low set ears, supernumerary nipples etc.) in about 7% of the children and a combination of major and minor in about 1% of the cases; of the major malformations, about 42% were multiple and

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1 Those that can be diagnosed easily and unequivocally within the first six days of life: anencephaly, spina bifida, hydrocephaly, cleft palate, cleft lip ± palate, oesophageal atresia, rectal atresia, hypospadias, reduction deformities of the limbs, congenital hip dislocation and Down's syndrome.

2 The mention of racial or ethnic groups reported here or elsewhere in this report does not imply that the United Nations accepts or recognizes such categories.
of the minor malformations, about 25% were multiple; the cardio-vascular system had the highest frequency of multiple malformations (about 80%); (ii) the highest incidence of malformations was found among neonatal deaths and deaths which occurred during the first year of life; (iii) males had significantly more malformations than females and this was entirely due to an increase in the frequency of major malformations; and (iv) there were no significant differences in the overall frequencies of major malformations between "non-whites" and "whites", but the "non-whites" had significantly more minor malformations than "whites", largely due to an increase in "non-whites" of polydactyly, branchial cleft anomalies and supernumerary nipples; the frequency of multiple malformations was significantly higher in "whites" than in "non-whites".

10. Leck [L2] summarized the estimates on prevalence rates at birth for several potentially lethal or handicapping malformations in the United Kingdom (excluding Down's syndrome; data derived from different sources; see Leck [L2] for details). For this purpose, data on cardiac malformations, pyloric stenosis, non-postural talipes equinovarus, hydrocephaly, penial and perineal hypospadias and many others were included. Conditions of minor importance (i.e., those that cause no appreciable handicap or threat to life) such as accessory auricle, glandular hypospadias, most postural foot deformities, polydactyly, syndactyly, etc., were excluded. The overall estimate is 24.4 cases per 1000 total births. In terms of severity (expressed as percentage alive five years after birth), about four-sevenths of the children with cardiac malformations (prevalence at birth: 6.5 per 1000) and two-thirds of all the malformed survive up to that age.

11. Leck also examined the transmissibility of the common malformations mainly using figures on frequency in sibs and offspring of index patients published by Carter [C6]. These data, summarized in Table 1, show that the percentages of first-degree relatives affected (in each case by the same malformation as the index patient) all lie between 2 and 5%, except for pyloric stenosis and hip dislocation (which are very much commoner in one sex than in the other); the recurrence risk seems to be higher when the index patient is of the less frequently affected sex. These findings are consistent with the hypothesis that the aetiology of each of the common defects involves a multiplicity of causes, some genetic and some environmental. The rates are all much lower than expected if single genes with complete penetrance for major effects were involved.

12. Altukhov [A1] compared the rates of occurrence of rare electrophoretic variants (i.e., those with an altered electrophoretic mobility or activity) in normal healthy newborns (group a), in premature infants and in babies with multiple congenital malformations (group b). The sample sizes for groups (a) and (b) were, respectively, 504 and 227, although the number of individuals screened for any given enzyme varied from 156 to 504 (a) and from 82 to 227 (b). Over twenty genetic loci coding for the synthesis of enzyme proteins and erythrocyte antigens were studied. Five variants were found in group (a) and 15 in group (b). As it was possible to exclude familial variants, it would appear that a much higher rate of incidence of these variants in premature infants and in those with multiple congenital malformations is of significance.

13. Recently, there has been considerable discussion by Neel [N1, N2, N3] on the contribution of mutation to ill-health in man. He has argued that current and anticipated developments in research are likely to lead to a significant upward revision of the UNSCEAR [U1] estimates of the frequencies of dominant, sex-linked and chromosomal diseases; and, more importantly, that the "UNSCEAR report probably grossly underestimates the contribution to disease of mutations which technically must be classified as "recessives". The latter argument rests on the following considerations.

14. The existence of a wide range of diseases known to be due to the absence of activity of an enzyme or of a transport or receptor protein is now amply documented. For instance, many of the classical "inborn errors of metabolism" (reviewed in [H1, K1]) are associated with the absence or near-absence of enzymatic activity as are some of the inherited bleeding disorders, thyroid disorders (reviewed by [S2]) and a class of non-spherocytic haemolytic anaemias due to the absence or near-absence of enzymes such as pyruvate kinase, hexose kinase, glucose-phosphate isomerase, etc. (reviewed by [M1]). The defects in DNA repair mechanisms now known in the various forms of xeroderma pigmentosum, ataxia telangiectasia and Fanconi's anaemia are the result of enzyme deficiencies. Familial hypophosphatemic rickets. Hartnup disease and a dozen other rare entities are probably due to the absence or malfunction of a transport protein (reviewed in [S3]). And testicular feminization and the severe form of familial hypercholestrolaemia are due to absence or malfunction of a receptor protein [A2, G1, M2]. These defects are all due to what in the past have been termed "null" mutations, usually with, in classical terminology, a recessive form of inheritance although heterozygotes when properly studied show impaired activity as well. Since it is difficult to envisage heterozygote advantage for these null mutants, it seems likely that most, if not all, are maintained by mutation pressure.

15. A rough idea of the magnitude of the total impact of this type of mutations can be arrived at through the following lines of reasoning. Based on a study of five enzymes in Drosophila, Mukai and Cockerham [M3] estimated that null mutations (characterized by the loss of enzyme activity) arise at a rate of 1.0 10⁻⁵ locus/generation whereas electrophoretic variants arise at a much lower rate (0.2 10⁻⁵ locus/generation) giving a ratio of 5:1 for electrophoretic variants. In Amurians, Neel et al. [N4] estimated that mutations resulting in electrophoretic variants of a series of proteins of the blood serum and erythrocytes occur at a rate of 1.6 10⁻⁵ locus/generation. If it is assumed that the rate in man is only 1.0 10⁻⁵ locus/generation for electrophoretic variants, and if the ratio of nulls to electrophoretic variants is only 2, then null mutations for a given polypeptide should be expected with a frequency of 2 10⁻⁵ locus/generation.

16. In man, there must be at least 5000 proteins whose absence or failure of function can lead to diseases such as those mentioned in the preceding paragraphs. Assuming no allelism, equilibrium between input and output and the stability of gene number.

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For instance, McKusick's 1978 catalogue [M16] lists 736 proved autosomal dominant (+753 probable ones); 521 proved autosomal recessive (+496 probable ones) and 107 proved X-linked (+98 probable ones) diseases in man. In the 1975 catalogue [M17] the respective numbers were: autosomal dominants: 583 (+635); autosomal recessives: 466 (+481) and X-linked ones: 93 (+78).
loss of mutants in the population, no heterozygous advantage or disadvantage and neglecting linkage, the probability that a zygote would be homozygous for at least one of these is \((1 - 0.99998^{100})\) or 0.095. While there is no doubt that this estimate needs to be viewed with many reservations, it does serve to illustrate the point that the impact on health in toto of this class of mutations may exceed the commonly visualized gross phenotypic abnormalities.

17. To what extent null mutations contribute to foetal loss in humans is not known. In Drosophila, null homozygotes or null/deficiency heterozygotes at 12 out of 13 autosomal loci studied are viable and fertile [O1, O2]. Neel points out that this is certainly not the case with all null mutants in humans and that the apparent difference between Drosophila and man may be an artifact, i.e., flies with the degree of impairment experienced by many of the human null homozygotes are probably so inviable as to preclude scoring. He speculates that the null homozygotes may make a significant contribution to the relatively high foetal loss in humans, but the demonstration that as much as 50% of recognized foetal losses are due to one or another kind of chromosome anomaly [C4] "preempts a large part of that selection arena" [N1, N2, N3]. While the proportion of null homozygotes that survive to term is not known, it is probably a sizeable fraction. Furthermore, since the accumulation in the gene pool of null mutations should facilitate the expression of classical recessive genes, one cannot, by invoking intra-uterine selection against most of them, dismiss the nulls as scarcely contributing to the social burden of mutation [N1, N2, N3].

18. Carter [C51] who recently examined the above arguments and their implications, points out that the thesis that almost 10% of foetuses are homozygous for a null mutation and that a substantial proportion of null homozygotes may survive to term is difficult to reconcile with the current stillbirth, infant and childhood mortality figures which in some areas are each well below 1% and the majority of which are caused by prematurity and congenital malformations, which are not recessive conditions: spontaneous abortions which occur at about 150 per 1000 recognized pregnancies offer more scope, but, as Neel notes, as many as half of these are due to chromosomal abnormalities, and others will have purely obstetrical causes. Neel's estimates imply that, on the average, every individual is heterozygous for about 50 null mutants; this is also difficult to reconcile with the health of most children born to first-cousin marriages, since zygotes conceived in such marriages, would be homozygous for one to two null mutants on the average; furthermore, first-degree incestuous unions (where zygotes would be, on the average, homozygous for six null mutants) would be almost incapable of producing viable children and this is not the case. As Carter stated, if "recessive" null mutations do occur with the frequency Neel suggests, then "... one must assume that their gene frequency is kept low in the population by selection against them in heterozygotes and ... are best classed with the dominants" [C51].

C. NUMERICAL AND STRUCTURAL CHROMOSOME ABNORMALITIES

1. Surveys of newborn children

21. In its 1977 report, the Committee discussed the results of surveys carried out in different parts of the world on the cytogenetic analysis of chromosomes in peripheral blood lymphocytes of liveborn infants. The data available at that time showed that out of 5679 babies examined, 336 (0.60%) had abnormal chromosome constitutions (pooled data). The breakdown of the above frequency was: 0.22% sex chromosome anomalies; 0.14% autosomal trisomies; 0.19% autosomal structural euploid abnormalities (Robertsonian and reciprocal translocations) and 0.5% autosomal structural aneuploid anomalies. The karyotype was examined with conventional staining techniques except in the Hamilton Survey [L1] in which banding methods were used.

22. The compilation of Hook and Hamerton [H3] focuses attention on consecutive newborns only, and includes additional data from the Boston survey [W2] but excludes the data of Lin et al. [L1] and those of Bockklov et al. [B1] (these were included in the 1977 report). The basis for exclusion of the data mentioned above was the use of banding techniques in the work of Lin et al. (and conventional techniques in the case of others) and the possibility of some selection in the study of Bockklov et al. However, the frequencies of chromosomally abnormal infants recorded in these two surveys (0.48% in the work of Lin et al. and 0.76% in that of Bockklov et al.) fall within the range of frequencies recorded in the other surveys (0.48% in London, Canada; 0.47% in Winnipeg, Canada; 0.63% in Aarhus, Denmark; 0.67% in Edinburgh, United Kingdom;
0.61% in Boston, United States and 0.50% in New Haven, United States).

23. Buckton et al. [B20] and Maeda et al. [M53] have now published the results of other newborn cytogenetic surveys carried out in Scotland and Japan, respectively (referred to as Edinburgh-UK-II and Kanagawa, Japan in Table 2). The Edinburgh survey includes: all babies born alive in one of the Edinburgh hospitals sampled previously [T2]; and all babies born alive in 1976 and most of 1977 in a maternity hospital in the Fife region. A total of 3993 babies were karyotyped of which 3835 could be analysed using G-banding. The Japanese data pertain to 2626 consecutive newborns screened in one hospital. Banding techniques were used. The data from these studies are summarized in Table 2. Not included in this table are the data of Turner and Wald [T3] and those of Higurashi et al. [H36, H48]; the reasons for this will be discussed later.

24. It can be seen that of the 424 (0.63%) chromosomally abnormal infants, 158 (about one-third) carry sex-chromosomal anomalies. There are 95 (about one-quarter), numerical autosomal anomalies; there are 134 (about one-third), balanced structural anomalies4 and 37 (about one-tenth), unbalanced structural anomalies. The incidence of sex-chromosomal anomalies alone is 3 per 1000 male births and 1.5 per 1000 female births. Of the autosomal numerical anomalies (1.4 per 1000 births), the +G anomalies (Down's syndrome) constitute the predominant group. Among the balanced autosomal structural anomalies (2 per 1000), reciprocal translocations and Robertsonian translocations are about equally frequent; among the latter, those involving two D group chromosomes are more common (48 out of 60) than those involving D and G group chromosomes. Finally, aneuploid structural abnormalities occur at a frequency of about 0.6 per 1000 births.

25. Turning now to results not included in Table 2, the work of Turner and Wald [T3] on newborns at Magee Women's Hospital in Pittsburgh was carried out between 1962 and 1964 and was published only in 1970. In this study which involved 1000 infants (517 males and 483 females), very strict attention was paid to randomization: neonates born were selected for study by the use of a random sampling frame in which one of the six four-hour time periods of each of the first four days of the week was chosen by random numbers; the first four deliveries of the selected periods were studied. Details of other aspects of the randomization procedures are given in the paper of Turner and Wald [T3].

26. The number of chromosomally abnormal babies in the above study was 33 (i.e., 3.3%; 2% with sex-chromosomal anomalies and the remainder with autosomal anomalies). This frequency is about five times higher than that reported by others and different from that of any other single study (see Table 2). The increase was in all types of abnormalities with the exception of 47, +21. Hook and Hamerton [H3] have suggested several possible reasons for this discrepancy, but none appear to be satisfactory and no further information from this study appears to be available.

27. The design of the study of Higurashi et al. [H36] on the incidence of chromosome anomalies in newborns in a Tokyo maternity hospital differed in several respects from those of others. Firstly, cytogenetic analysis of the karyotypes was not carried out for all the babies. The babies were first screened for clinical manifestations during the first day of life; a buccal smear was obtained for an examination of both X and Y chromatin (primary screening method). A secondary screening included repeated buccal smears and lymphocyte analysis for Y chromatin after two or three months and this was done to ensure that there were no false positives or negatives. All suspected cases were examined by detailed chromosome analysis. Secondly, babies thought to have congenital malformations were re-examined and this included a study of their dermatoglyphic patterns (in the experience of the authors, malformations associated with mental retardation and abnormal dermatoglyphic patterns were strongly suggestive of autosomal aberrations [H37]). Those judged abnormal by the criteria of malformations, dermatoglyphic patterns, low birth weight (and history of abortions in the mothers) were then examined through analysis of karyotypes.

28. Of the 3311 phenotypically normal male babies studied through analysis of sex chromatin, 31 were suspected of possibly carrying sex-chromosomal anomalies; chromosome analyses of these showed two cases of 47,XXY, two of 47,XYY and one of 46,XY,-D5 (Dp,Yq). In 2054 phenotypically normal female babies likewise examined, sex-chromosome anomalies were suspected in 21 and out of the latter, one had a 45,X karyotype.

29. For autosomal anomalies, the total number of babies initially screened was larger. Of the 12 319 babies (total sample), 694 were suspected of having autosomal anomalies (353 males and 341 females). Of the 694, two were +13, three were +18 (with one case of mosaicism), eleven were +21 (with one case of mosaicism), one with 45,−5, partial trisomy and one which was 5p− (cri-du-chat syndrome). The total frequency of chromosomally abnormal individuals in this study (25 in 12 319) cannot be readily compared with those obtained in other studies because the design is different; this is true also of some individual classes (sex-chromosomal aneuploidies, autosomal trisomies).

30. The other study of Higurashi et al. [H48] was focused on ascertaining the birth frequency of multiple congenital anomalies in newborn infants (14 430 newborns out of which 7455 were males and 6975 were females). It was found that 33 of the babies had multiple congenital anomalies. The birth frequencies of the three major trisomies (13, 18 and 21) were, respectively, 0.14, 0.21 and 1.11 per 1000 births, in good agreement with those recorded in the other surveys listed in Table 2. The authors point out that most cases of sex-chromosomal anomalies remained undetected as these showed no obvious manifestations at birth.

2. Clinical significance of chromosome abnormalities

31. Hook and Hamerton [H3] used the data pertaining to the 56 952 babies reviewed in their paper to estimate the frequency of "clinically significant" abnormalities. The authors included in this category all the non-mosaic XXY and 45,X instances, XXY and XX (male) genotypes, all autosomal trisomies and all unbalanced structural rearrangements reported to have been
associated with congenital malformations at birth. The balanced structural abnormalities and sex-chromosomal mosaics were excluded. The reasons for the exclusion of the latter were: (a) the variation in ascertainment between the studies; (b) the lack of information on the extent of abnormalities in the tissue concerned; and (c) the inability at present to decide about the clinical significance of mosaicism detected in peripheral blood lymphocytes in phenotypically normal babies.

32. The estimate arrived at is 2.91 per 1000, i.e., about one-half of the total of all chromosomal anomalies detected in newborns. If the XXY and XX (male) karyotypes are excluded, the rate is 2.27 per 1000. An analysis by sex of those affected indicated that the rate in male newborns is about twice as high as in females primarily because of the unequal contribution of the sex-chromosomal abnormalities judged by these authors as "significant". If one excludes the XXYs and XX males, the rate is still 60% higher in males.

33. An extension of the criteria used by Hook and Hamerton to the total data given in Table 2 gives a rate of about 3.3 per 1000 for the abnormalities that may be deemed to be "clinically significant", again about one-half of all the abnormalities detected in newborns. If the XXY and XX (male) genotypes are excluded, the rate is 2.6 per 1000. These figures are in good agreement with those reported by Hook and Hamerton.5

34. There are several reasons why the estimates arrived at in paragraphs 32–33 may be underestimates. Firstly, only long-term follow-up studies will provide definitive answers to the question of what proportion of the anomalies may have clinical significance. While it is true that the clinical significance of certain chromosomal conditions such as trisomies for chromosomes 21, 13 or 18 can be relatively easily diagnosed at birth, this is by no means true for all abnormalities. Secondly, the frequency of sex-chromosome mosaics detected in many newborn surveys may be lower since the number of cells analysed may not be adequate and, to determine the extent of mosaicism, more cells need to be analysed. Furthermore, as Hook and Hamerton have pointed out, it is difficult to assess the clinical significance of mosaicism from chromosomal analysis of peripheral blood lymphocytes of phenotypically normal individuals. Probably, at least some individuals with 45,X/46,XY 45,X/46,XX or related karyotypes may eventually prove to be of clinical significance, but this depends on the magnitude of mosaicism and the tissues involved. Only long-term follow-up of individuals detected in such surveys may provide clear-cut answers.

35. Thirdly, data showing that apparently balanced or euploid structural rearrangements (translocations and inversions) in man can have deleterious phenotypic effects (and thus assume clinical significance) are slowly accumulating. Jacobs [11] examined the data from the Edinburgh survey of the general population, of newborns and of mentally subnormal groups and found a significantly higher proportion of "mutant" balanced rearrangements in the mentally retarded group (excluding Down's syndrome) than in the others (5 out of 7 rearrangements in the mentally retarded group were of de novo origin, whereas in the other two groups combined the respective figures were 7 out of 9; see also [1] for a recent discussion of these data). Breg's updated results [21] on chromosome analysis of the mentally retarded at the Southbury Training School in Connecticut [32] show a similar situation: a total of 9 balanced structural rearrangements (1 Robertsonian translocation, 6 reciprocal translocations and 2 inversions) were detected in 1087 individuals. In this study, however, the relative proportions of familial versus de novo rearrangements were not ascertained.

36. Other clinical data come from the work of Tharapel et al. [14], Funderburk et al. [11], Aurias et al. [3] and Fryns and van den Bergh [2]. Tharapel et al. [14] found 6 cases of de novo apparently balanced reciprocal translocations which were associated with mental retardation and multiple congenital abnormalities (5 cases) or with ambiguous genitalia and multiple congenital anomalies (1 case). Funderburk et al. [11] reported that the incidence of balanced chromosome rearrangements was higher among mentally retarded children (7 in 435) than among patients with psychiatric disorders (4 in 1679). In the latter group, all the four were pericentric inversions. The authors point out that Aurias et al. [3] show that among 2341 children with malformations and/or mental retardation, 13 had balanced reciprocal translocations (of which 7 were familial), 5 had Robertsonian translocations and 2 had pericentric inversions. In addition, in 762 children with trisomy 21, three familial balanced reciprocal translocations were observed and none of these affected chromosome 21.

37. The studies of Fryns and van den Bergh [2] on lymphocytes of 12,160 patients (using currently available banding techniques) showed that 32 of these patients were carriers of apparently balanced, reciprocal autosomal translocations. Eleven out of the 32 were detected in patients with mental retardation and/or some malformation. In 7 of these, the translocation was familial; in one newborn with multiple congenital anomalies, one of the parents could not be karyotyped and in the remaining 3, the translocations were of de novo origin. The authors point out that while the occurrence of mental handicap with or without congenital anomalies in patients with a de novo translocation may be explained as due to the possible occurrence of a deletion (during the formation of the translocation) which may be undetectable by present techniques, it is hard to envisage such a situation in the case of familial translocations.

38. Data bearing on the clinical significance of chromosomal anomalies are also being collected in the Collaborative Perinatal Project (CPP) mentioned earlier. The paper of Patil et al. [1] summarizes some of the main findings from cytogenetic studies carried out on the children when they were 7 or 8 years old in five of the twelve centres involved in the study (these children have extensive neurological, developmental, psychological and other clinical and family data systematically recorded from birth onwards without knowledge of their chromosome constitution and therefore the cytogenetic study of the 7 or 8 year old children is an unbiased one).

39. A total of 4342 children (2156 females and 2186 males) were examined and 21 (0.48%) showed major chromosomal anomalies. There were 8 translocations, 3 pericentric inversions (1 autosomal and 1 each in an X and Y chromosome), 2 trisomy 21's, 3 X chromosome mosaics, 2 other X chromosome aberrations and 3 XYYs. Of the 8 translocations, 3 were balanced Robert-
sonian and the rest balanced reciprocal ones. The frequency of sex-chromosomal anomalies in females was 0.23% (5/2156: 2 mosaics and 3 structural anomalies) and in males, 0.23% (5/2186: 1 pericentric inversion of the Y, 1 mosaic (47,XXY/46,XY) and 3 XXXs). No partial autosomal monosomies or trisomies were detected. Table 3 in which the overall frequencies observed in the CPP are compared with those in newborn surveys shows that these are in good agreement except in the case of autosomal trisomies. The lower frequency of these in the CPP results is presumably due to the fact that infants with trisomies 13 and 18 and some of the children with trisomy 21 died (or in the latter case were institutionalized) and consequently were not included in the sample.

40. Six of the children carrying translocations (although of normal weight and length at birth) had minor clinical problems including clubfoot, reading disability, abnormal hearing and an abnormal skull shape. Three of the children who had de novo translocations had at least one of the above-mentioned abnormalities. All the five chromosomally-abnormal female children (including mosaicism) had one or another kind of problems such as mental deficiency, neurological abnormalities, abnormal speech and motor development, small height and weight throughout development, and so on.

3. Chromosome anomalies in perinatal deaths

41. Only a few studies [A4, B4, K2, M4, S5, S86] have so far been carried out to examine the prevalence of chromosomal anomalies in perinatal deaths (i.e., those babies who die before or during delivery or during the first week). The frequency of anomalies have been estimated to be of the order of 5–6%, this being higher among macerated stillbirths than in fresh stillbirths and in early neonatal deaths [A4]. The recent compilation of results from four different centres [S86] shows that the frequency of chromosomal abnormalities in macerated stillbirths is 11.6% (13/112) dropping to 3.8% (13/340) in non-macerated stillbirths and to 5.0% (41/924) in early neonatal deaths. Among the anomalies recorded, trisomies predominate, particularly those involving chromosomes of group E, followed by structural anomalies, triploidy and others.

4. Chromosome anomalies in spontaneous abortions

42. The incidence of chromosomal anomalies in spontaneous abortions in humans was extensively reviewed in the 1977 report. The recent summary of results presented by Carr and Gedeeon [C4] and other papers published in the literature [H4, H49, K29] support and extend the conclusions of the Committee given in the 1977 report, which follow:

(a) The overall frequency of chromosomal anomalies among spontaneous abortions may be as high as 50% when corrections are made for non-hospitalized patients and for undetected induced abortions;

(b) Trisomies as a group constitute the most common accounting for about 50% of all chromosomal anomalies among abortuses, followed by monosomy-X (18%); triploidy (17%); tetraploidy (6%) and others (7%; includes double trisomies, mosaics and structural rearrangements);

(c) Trisomies for all members of the chromosome complement have been found among abortuses except those for chromosome 1 and 5, although the relative involvement of the different chromosomes is different; thus, for instance, trisomy 16 is clearly the most common amounting to 30% of all trisomies; trisomies for chromosomes 6, 11, 12, 17, 19 and X are rare; trisomy 21 and 22 have approximately equal frequencies (10% each);

(d) Unbalanced translocations account for about 2 to 4% of all abnormalities observed in foetuses and this frequency is much higher than among liveborns.

43. Evidence for the maternal age-dependence for trisomies involving acrocentric chromosomes (D and G groups) was discussed in the 1977 report. The more recent results of Hassold et al. [H50] document and extend the earlier findings. In this study in which data from 362 trisomic and 790 chromosomally normal spontaneous abortions were compared with respect to the age of the mothers, it was found that trisomies as a group were associated with a substantial increase in maternal age, although there were considerable differences in the magnitude of the effect between different trisomies. The effect of maternal age was most pronounced for trisomies involving the small chromosomes, both acrocentric and non-acrocentric. Trisomy 16 was conspicuously different from trisomies for all other small chromosomes, both in the reduced importance of increased maternal age and in the high frequency with which it occurred. The effect of increasing maternal age on trisomies for chromosomes in groups A, B and C was less clear than for the small chromosomes.

44. Hassold et al. [H50] speculate that the maternal age-dependent trisomies may result from precocious disjunction of the bivalents and random segregation of the resulting univalents, a process which would affect chromosomes with the fewest chiasmata and which might be more prevalent in oocytes of older women. They further suggest that true non-disjunction (i.e., the failure of bivalents to separate at anaphase) may also result in the production of trisomies and that this process may be independent of, or only slightly influenced by, increasing maternal age, but be affected by the presence of large blocks of heterochromatin.

45. With the advent of banding techniques to study human chromosomes, it became clear that several chromosomes contain heteromorphic regions which are inherited like Mendelian genes and that these heteromorphisms are frequent in the acrocentric chromosomes. Applied to trisomic cases, these markers provide a powerful tool to ascertain, in favourable situations, in which sex and at which meiotic division non-disjunction has occurred. This has been done for trisomies for certain chromosomes in abortus material and in the case of Down's syndrome.

46. The data summarized by Jacobs and Hassold [J15] given in Table 4 permit the following inferences: for trisomy 16, non-disjunction can occur at any one of the meiotic divisions in either sex with meiotic I error in the female predominating; for others (trisomy 13, 14, 15, 21 and 22), non-disjunction seems to occur almost exclusively at division I in the female. The predominance of meiotic I errors in the female for both age-related (D and G group chromosomes) and in apparently age-unrelated (trisomy 16) is of importance in considering the mechanism of non-disjunction (see also [F21]).
5. Mutation rates

47. The 1977 report presented estimates of mutation rates for the different kinds of chromosome anomalies that result in liveborn children. For numerical errors of autosomes, the estimated rates were 7.5 $10^{-4}$/gamete/generation for sex-chromosomal errors and 6.7 $10^{-4}$/gamete/generation for autosomal errors (giving a total rate of 14.2 $10^{-4}$/gamete/generation). The estimates based on the results summarized in Table 2 are nearly the same, being 8.3 $10^{-4}$/gamete/generation for sex-chromosomal errors and 6.9 $10^{-4}$/gamete/generation for autosomal errors (giving a total of 15.1 $10^{-4}$/gamete/generation).

48. The earlier estimates [U1] for balanced structural rearrangements of autosomes and for unbalanced rearrangements were, respectively, 1.9 $10^{-4}$/gamete/generation and 0.45 $10^{-4}$/gamete/generation. In two recent papers, Jacobs [J3, J16] has given revised estimates based on the data for 48,650 and 59,452 babies, respectively. Much of the information on which these estimates are based overlaps with that given in Table 2 and, consequently, these rates may be considered to reflect our current state of knowledge in this area. Jacobs [J16] has also presented some estimates based on the incidence of structural (balanced and unbalanced) chromosomal anomalies in spontaneous abortions. These, taken from her more recent paper, are summarized in Tables 5, 6 and 7.

6. An overview of the importance of aneuploidy and structural aberrations

49. Three recent papers have extensively dealt with different aspects of spontaneously arising aneuploidy and structural aberrations including their contribution to foetal wastage and genetic ill-health in humans [C52, F21, S6]. An overall perspective of their relative contributions can be gained by relating the incidence frequencies recorded in new-born surveys, perinatal deaths and in spontaneous abortions. Figure 1 taken from the paper of Sankaranarayanan [S6] and modified to take into account the frequencies of different kinds of anomalies discussed in this Annex provides a summary of these data. In drawing this figure, it has been assumed that the level of spontaneous abortions is of the order of 15% and that 2% of the children die perinatally. Some other important aspects of aneuploidy and structural aberrations in humans, not covered in Figure 1, will be briefly dealt with in the following paragraphs.

50. For monosomy-X, there is indirect evidence that maternal meiotic non-disjunction may not be the main underlying cause. It has been shown [C53, K30, W23] that maternal age is not elevated in the mothers of XO conceptuses; in fact, the incidence of XO appears to be highest among young women, a finding which suggests either that there is an increase in events leading to meiotic or early cleavage errors in younger women, or possibly that a greater proportion of their XO conceptuses survive to a stage of becoming recognizable pregnancies [W23].

51. For women with Turner's syndrome, Sanger et al. [S87] have estimated that 77% of the preovum have a maternal X chromosome. Garron and Lindsten [G34] found that X monosomic patients have, on the average, more brothers than sisters. This finding suggested to the authors that the X chromosome is more often lost during spermatogenesis than the Y chromosome. Paternal sex-chromosome loss from XX or XY zygotes at early cleavage could also play a role in the genesis of X monosomy in humans.

52. With respect to their reproductive potential, the chromosomally abnormal types can be roughly divided into four major categories [C52]: the viable steriles (XO, XXY conditions, all male and some female reciprocal X-autosome translocation heterozygotes, Y-autosome translocation heterozygotes and some purely autosomal translocation heterozygotes), viable semi-steriles (most balanced structural heterozygotes, mainly the translocation and inversion carriers), viable but non-repro-

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Figure 1. A summary of the estimates of chromosome anomalies (per 10⁴ conceptions) in spontaneous abortions, perinatal deaths and in children. Modified after [S6]

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ductive types (viable trisomy 21 individuals and viable carriers of unbalanced structural rearrangements) and viable fertile types (XXX and XYY conditions). A recent workshop [B78, S91] was devoted to chromosomal aspects of male sterility in mammals including humans.

53. The defect in XO women and XXY men appears to be related to germ cell survival and sex-chromosomal make-up [B61]. Germ cells are present in the ovaries of human XO foetuses [S88], but loss begins late in foetal life and by birth few oocytes remain [C54]. The generally observed sterility of XXY males both in man and in many other mammalian species investigated in this respect has led to the postulate that the presence of two X chromosomes in a testicular germ cell results in its perinatal death [B61]. In female carriers of balanced reciprocal X-autosome translocation, the risk of infertility seems to be brought about by gonadal dysfunction in a proportion of the cases. Summitt et al. [S89] reviewed the literature on this subject and found that each female carrier in which the X breakpoint falls between bands Xq13 and Xq27 is liable to show infertility, with primary amenorrhoea in most cases, while carriers with X breakpoints in other regions are fertile.

54. In male carriers of an X- or Y-autosome reciprocal translocation, and in some cases of autosome-autosome translocations in humans [C55, C70] as in the mouse [S90] the infertility seems to be associated with spermatogenic arrest. For the purely autosomal reciprocal translocations, those with one point of exchange close to the centromere and the other fairly distal seem to be associated with infertility [C55] and this is true also in the mouse [C56, S90, S92]. In humans, among the cases so far described, an acrocentric chromosome frequently seems to be involved in such male-sterile translocations [C55]. The sterilizing effects of these autosome-autosome translocations in both mice and humans appears to be limited to male heterozygotes. In humans, where the translocation has been shown to be familial [B62, C57, L39], sterility and azoospermia have been reported in more than one male heterozygote, but no effects on gamete production in females carrying the same translocation have been apparent.

55. A sizeable proportion of structural heterozygotes are effectively semi-sterile since these individuals produce gametes carrying unbalanced segregation products that can lead to zygotic loss, spontaneous abortions and birth of congenitally malformed children. Each individual translocation, however, is likely to be unique with regard to the level of imbalance produced and its consequences. Very little is known about the reproductive potential of unbalanced translocation heterozygotes, but where meiotic studies have been performed on occasional males, they have shown severe impairment of the spermatogenic sequence [F22].

7. Progress in work with Down's syndrome

56. General considerations. Down's syndrome is the best known autosomal aneuploidy in man on which a large amount of literature exists (see [B10, L3, P5, S16, S93] for comprehensive reviews). The incidence is between 1 and 2 per 1000 births among various populations [H6, H7, H8, K31, K32, L40, M5, M54, M55, N22, N94, T1]. Three cytogenetic types of Down's syndrome are known: trisomy 21, translocation and mosaics. Trisomy 21 accounts for over 95% of the cases; about 2-5% are due to translocation, the most frequent being a translocation of a chromosome 21 with a D or G group chromosome. Chromosome 14 is the one most frequently involved in the D group [H5] although chromosome 15 is also rarely involved [N6]. G/G translocations are either t(21q21q) or t(21q22q). About 50% of the D/G and 90% of the G/G translocations are de novo ones, and the remainder are familial. Between 1 and 2% of Down's syndrome patients are mosaics, the most common being 46/47, +21.

57. Although there are no clinical differences between patients carrying the standard trisomic type and those with translocations, there are some important differences regarding their actual or potential family history. Rare exceptions apart, the standard trisomic condition arises by non-disjunction during gametogenesis in one of the parents, more often in the mother. It has been known for a long time that the risk of bearing a child with Down's syndrome increased with advanced maternal age. After the parents have had one child with Down's syndrome the risk of recurrence appears slightly elevated above the risk for the general population. Carter and Evans [C18] estimated the recurrence risk in Britain as 1-2% irrespective of maternal age.

58. If chromosome 21 is translocated to another chromosome, the situation is different because such translocations may be transmitted from generation to generation in the balanced state. The actual risk of producing a child who is effectively trisomic varies, depending on which chromosomes are involved and on whether the father or mother is the translocation carrier. As stated earlier, the majority of D/G translocations involve chromosomes 21 and 14 and it has been estimated on the basis of family data that a mother who carries the translocation has a 10% chance of producing a child with Down's syndrome; if the father is the carrier of the balanced translocation, the risk of Down's syndrome is thought to be only 2-3%. These estimates, however, are preliminary and it is likely that the real risk of having children with Down's syndrome may be higher at least in some families [M6].

59. The recurrence risk of Down's syndrome in families with a G/G translocation also varies depending on the sex of the carrier and on the chromosomes involved. Although the data are not extensive, in the case of a t(21q22q), the female carrier has about 9% chance of producing a child with Down's syndrome. Reliable data are not available for carrier fathers, but the risk appears to be small [M7, S9]. With a t(21q21q), the risk is 100% irrespective of the sex of the carrier, since all gametes will be either disomic or nullisomic for chromosome 21, which means that all children surviving to term will be affected.

60. Parental origin of the extra chromosome in trisomy 21 and in translocation Down's syndrome. In the first publication using chromosome heteromorphisms to study the origin of the extra chromosome 21, Licznierski and Lindsten [L4] traced it to the mother. Since then, a number of studies have been conducted [B11, M9, M10, M56, L5, R2, S10, U2, W4]. These studies have established that the extra chromosome can be either maternal or paternal in origin and furthermore, that the disjunctional errors arise in either of the two meiotic divisions. Verma et al. [V2] compiled the results of banding analysis of 67 "informative" cases of Down's syndrome and their parents. Non-disjunction was maternal in 44 cases (65.7%) and paternal in 23 cases.
In 37 of the 44 maternal-origin cases, non-disjunction occurred in meiosis I and in the remainder, in meiosis II. In the paternal origin group, in 14 cases, it occurred in meiosis I and in the remaining 9, in meiosis II. These conclusions are similar to those of Langenbeck et al. [L5] and of Magenis et al. [M19].

61. In a more recent paper, Mikkelsen et al. [M56] have compiled data from the literature (including some of their earlier ones) in addition to presenting some new data for two areas of Denmark, on the parental origin of trisomy 21. Considering first the literature compilation, out of 145 informative cases, the extra chromosome was maternal in origin in 112 (72.8%) and paternal in origin in 33 (22.8%). In the first group, 96 out of the 112 cases (86%) were due to division I errors, the remainder being due to division II errors. In the second group, 20 out of 33 cases were due to first division errors and the remainder due to second division errors.

62. The new data of Mikkelsen et al. [M56] pertain to Down's syndrome children born in the Danish islands of Funen and Zealand (the earlier paper of Mikkelsen et al. [M9] was on Down's syndrome in Zealand). The total material comprised 125 families with Down's syndrome children out of which 95 were informative (76%). In 77 children (81%), the extra chromosome could be traced to the mother and 18 (19%), to the father. In the new material alone, paternal failures were observed in 11% of the cases in Funen (5/45) and 23.5% of the cases in Zealand (8/34).

63. Jacobs and Morton [J4] have argued that the methodology used in many of the reports and/or the way in which the data are recorded, leads to the loss of much of the potentially useful information. For instance, Mikkelsen et al. [M9] and Wagenbichler et al. [W4] summarized their results, without giving the actual observations, for those "informative" cases for whom the origin of the chromosome(s) could be established. By not reporting all the observations, the authors have introduced a bias into their series and have made the calculation of the relative probabilities of the different mechanisms of origin of the extra chromosome difficult. By applying a maximum likelihood analysis to the data available to them, Jacobs and Morton [J4] reached the conclusion that "...it appears that trisomy 21 is due to first division maternal non-disjunction although there are case reports of second division non-disjunction, both paternal and maternal..." and suggested the need for complete reporting of the data and large systematic samples.

64. Data on the parental origin of translocation Down's syndrome are limited. The information summarized by Mikkelsen et al. [M56] and by Chamberlin et al. [C58] suggest that in about two-thirds to three-quarters of the cases the translocation was maternal in origin.

65. Maternal age. Maternal age has long been known to be an important factor in Down's syndrome (see [P5] and references given therein). This correlation between increasing maternal age and Down's syndrome applies to 95% of the cases which are primary trisomies for chromosome 21 and which result from non-disjunction [P5, M11]. For other types such as translocation Down's syndrome, both spontaneous and inherited, and for mosaics, no age-dependency has been proved [G2, R3]. Thus, among all the cases of Down's syndrome, there is a predominantly maternal-age-dependent and a maternal-age-independent class.

66. If the mother is less than 20 years of age at the time of conception, the risk of producing a child with standard trisomy 21 is about 1 per 2500 livebirths [P5, C7]. The risk gradually increases until 35 years of age, after which there is a more steep increase in the frequency such that a mother over 45 years of age has about 1 in 50 chance of having a child with Down's syndrome [P5, S11]. In more recent papers, Hook and Fabia [H6], Hook and Chambers [I17] and Hook and Lindsjö [H8] have analysed the data on the incidence of Down's syndrome in livebirths by single year intervals in maternal ages for, respectively, the State of Massachusetts (1958–1965), the State of New York excluding New York City, (1963–1974) and for Sweden. The main finding is that the increase in the incidence of Down's syndrome is gradual from maternal age 20 to about 30–31 and more pronounced thereafter. The effect of maternal age has also been confirmed by the results of the second Baltimore case-control study [C8].

67. In their 1980 paper, Lamson and Hook [L41] analysed the data published in the literature in which the rates for Down's syndrome had been given by 1-year maternal age intervals to define the shape of the curve describing maternal-age-specific rates. There were a total of 3613 Down's syndrome cases born to mothers of ages ranging from 20-49. Cases born to mothers below 20 years of age were excluded because the analysis suggested that some factors operative at ages 15–19 are not pertinent to rates at higher ages. Age 49 was chosen as the upper limit because of concern about the accuracy of reporting and coding of data for higher ages.

68. The statistical model used for analysis ("The constant plus exponential model" in the authors' terminology) is represented by the equation

\[ y = a + \exp(b + cx) \]

where \( y \) is the rate of Down's syndrome in live births, \( x \) is the maternal age and \( a, b \) and \( c \) are constants. The authors point out that the above model gives a good fit to the data "about as well as the approach that used separate equations", and that it does not postulate a sharp transition in biological processes around maternal age 30, but rather a process continuously accumulating at a constant exponential rate, superimposed upon a constant background rate. The equation predicts that there is one group of conditions that occur with a pooled constant rate a (maternal-age-independent?) and a second group of conditions that operate over the entire age-spectrum 20–49, its contribution increasing at an exponential rate with slope c (maternal-age-dependent?). They caution, however, that although their model may be more appealing statistically (and perhaps biologically as well, because its equation has fewer parameters), this does not imply that this model is necessarily more likely to be correct than the others. Furthermore, the calculations of the maternal-age-independent and dependent categories are consistent with the observed distributions, but are not the only possible inferences from them.

69. The effect of paternal age. Although it is widely accepted that the most important variable in the incidence of Down's syndrome is maternal age, the possible association with paternal age has also been considered. A number of investigations [L3, P5, S12], however, have failed to detect any significant effect of advancing paternal age in addition to that accounted for by maternal age.
70. Stimulated in part by a number of case reports on the paternal origin of the extra chromosome 21 detected through the use of the quinacrine banding techniques mentioned earlier, the search for paternal-age-effect has once again begun. Stene et al. [S13] have reported the results of an investigation conducted in the Copenhagen Metropolitan area to study the effects of paternal age on the incidence of Down's syndrome. Two hundred and twenty-four Down's syndrome patients born during the period 1960-1971 provided the material. The control was a random sample of 6053 births from the same period. By developing and applying a new statistical technique (detailed in [S14]), the authors found that: (a) there was an increasing incidence of Down's syndrome with advancing paternal age for a given maternal age; and (b) men above the age of 55 years have a significantly increased risk of begetting children with Down's syndrome. The Japanese data presented by Matsunaga et al. [M13] are supportive of the Danish findings: there was an excess of Down's syndrome infants born to fathers ≥ 55 years of age.

71. The regression analysis of Hook et al. [H51] of Down's syndrome cases born in 1964–1976 and reported by the British Columbia Registry for Handicapped Children showed that, after adjustment for maternal age effects, the data were consistent with an increase of about 1.024-fold for each year of paternal age throughout the entire age range (thus differing from the results of Stene et al. [S13] and Matsunaga et al. [M13] where the evidence was found only in men of 55 years and over). Among Down's syndrome cases for the period 1952–1963, however, for which ascertainment appears likely to be less complete, there was no evidence for a significant paternal age effect.

72. Erickson [E2] has published the results of an analysis of the data collected from 29 states and two large cities in the United States by the National Cleft Lip and Palate Intelligence Service (NIS) during 1961–1966. The data pertain to 4000 white infants with Down's syndrome and about 86,000 normal white babies. His analysis confirmed the maternal-age association with Down's syndrome with a high degree of statistical significance but did not demonstrate any independent effect of paternal age; in fact, the rates at paternal ages over 45 years appeared to be nearly the same. Erickson's conclusions, however, have been disputed by Stene and Stene [S15]. The latter authors have argued that the NIS data contain too few Down's syndrome cases with older fathers and thus are less suited for an investigation of a possible paternal age effect. Furthermore, they point out that the statistical tests used by Erickson have relatively low resolving power.

73. In a more recent paper Erickson [E3] examined again the question using two new sets of data derived from two sources: the Metropolitan Atlanta Congenital Defects Surveillance Programme and the National Centre for Health Statistics; the former pertained to the period 1968–1976 and the latter to 1972–1975. Analysis of these data (taking into account the criticisms of Stene and Stene [S15] and improving the methods of statistical analysis) showed however no overall excess of Down's syndrome infants born to older fathers in either case. The Atlanta data suggested an increased number of Down's syndrome infants born to older fathers who had children by a previous wife ≤ 34 years. There was a small deficiency of Down's syndrome infants born to older fathers by women ≥ 35 years. Erickson concluded that the possibility of a paternal age effect remains open, but the available data suggest that, if it exists, it is quite small.

74. Effect of radiation. In its 1977 report, the Committee reviewed the results of surveys designed to inquire whether parental (in particular, maternal) irradiation may increase the risk of producing Down's syndrome (see also [U3]). The main findings of all the retrospective and prospective surveys are recapitulated in Table 8 which includes also the recent results of the Baltimore Case-Control study [C8]. This study, however, in contrast to earlier findings [S19], found no significant effect of maternal irradiation on the incidence of Down's syndrome. (The Indian study [K4] has been excluded from Table 8 owing to problems of statistical analysis, as discussed in the 1977 report [U1]). Thus the only studies that show a positive effect are those of Alberman et al. [A5], Sigler et al. [S19] and Uchida et al. [U4, U5]. In conclusion, as Uchida [U6] stated "... it may still be premature to say with conviction that radiation as a cause of non-disjunction, increases the frequency of 21-trisomy. However, it seems logical to avoid unnecessary exposure to mutagens that might add to the genetic burden of humans".

8. Satellite associations: their possible functional significance and relevance for autosomal trisomies

75. The satellite regions of human acrocentric chromosomes (Numbers 13, 14, 15, 21 and 22) are frequently located near one another in metaphase chromosome preparations. This relationship called "satellite association" (SA) was first described by Ferguson-Smith and Handmaker [F3] and Harnden [H9] and has been found to occur both in mitosis and in meiosis [F4]. The length of the satellite stalk or secondary constriction, rather than the size of the satellite, is correlated with the frequency of associations [E4, S21, 21].

76. SA is thought to be the result of the involvement of the acrocentric chromosomes in nucleolus formation [F4, H10, O3] and the connectives between the satellite regions which can sometimes be observed in stained mitotic preparations [Z2] represent remnants of the nucleolus. Both the secondary constrictons of acrocentric chromosomes and the connectives have been shown to contain DNA (rDNA) coding for 18S and 28S ribosomal RNA (rRNA) [E4, H11, H12, C10]. According to a recent estimate [Y1] there are approximately 50 such genes, i.e., about 5 ribosomal genes on each acrocentric chromosome. It is recognized that the number of RNA genes on a given chromosome is variable [e.g., E4, D1, H12] and it has been suggested that such variations may be related to the frequencies with which particular chromosomes are found in satellite associations [H12, M15, W5].

77. The frequency of SA may vary within the population and a variety of in vivo and in vitro conditions (e.g., age, sex, thyroid autoantibodies, viral infection, chromosome culture methods, etc.) have been shown to have an effect on the frequency of SA (see Houghton [H16] for a citation of the relevant papers). There is a recent report showing that SAS are distorted when colcemid is used to collect metaphases [R64]. In part, interest in the problem of SA has been stimulated by the finding that the majority of the chromosomal abnormalities in liveborn children involve the acrocentric chromosomes and that these aneuploidies are
caused by non-disjunction and by structural rearrangements such as reciprocal translocations and Robertsonian translocations; and are also stimulated by the speculation that SA, if it occurs in germ cells, may have an actiological role in the development of these abnormalities [F3, H9, C11, C12, R5, O3, Z3].

78. Several workers have concluded that participation in SA is random [e.g., C13, C14, H18, J5, N7, R6, S22] while a number of others found that some particular associations were more frequent than others [e.g., C15, N8, A6, P7]. For instance, Cohen and Shaw [C13] found that there was a distinct tendency for G group chromosomes to be associated more frequently than D group elements; in multiple associations of specified size within a given number of D and G chromosomes, the type of associations appeared to be random. Jacobs et al. [J5] noted that there was very significant heterogeneity among individuals in the frequency with which different chromosomes entered into associations and were unable to obtain evidence for preferential association between any particular chromosomes, either homologous or heterologous. The existence of different and normal individuals has also been noted by a number of investigators [e.g., M15, H17, Z2]. On the other hand, Patil and Lubis [P7] found that the chromosomes most frequently involved in Robertsonian translocations (i.e., 14, 21 and 13) were also most frequently involved in satellite associations. The observations of Ardito et al. [A6] show that while the pattern of association of D-D, D-G and G-G groups seem to be random, there exists some preferential association particularly between pairs of 13-14, 13-13, 13-21 and 21-21.

79. The results of studies of Down's syndrome patients and their parents have also yielded conflicting results. Some studies have shown a significant increase in SA in blood cultures of Down's syndrome children [e.g., R7] and it has been reported that the parents of Down's syndrome children showed significant deviations from random association even though there was a random distribution for the children themselves [e.g., C12, R5]. Other workers could not demonstrate any difference between Down's syndrome patients, their relatives and normal controls [e.g., K37, T5]. Mok et al. [M18] studied SA patterns in a large sample of parents of normal children and parents of Down's syndrome children. In the latter group, more SAs and more complex associations were found. There were also significant differences in the composition of the association complexes: in parents of Down's syndrome children, chromosome 15 was less frequently involved than normal and all associations involving chromosome 22 were increased except for 22-15. For chromosome 21, 21-22 was more frequent than normal.

80. Houghton [H16] investigated the effects of gamma or neutron irradiation of blood derived from normal males and females, Down's syndrome males and females and the parents of both normal and Down's syndrome children on satellite association. In the range of gamma exposures employed (0.05 to 0.6 Gy) no effects on SA were apparent; the results obtained using the silver staining technique (which permits a more precise evaluation) were also the same. With neutrons however, while there was no change in the overall frequency of SAs, there was a change in the composition of the SA complexes: there appeared to be a preferential participation of chromosome 13 in SA over the dose range of 0.05 to 0.6 Gy. The author has no explanation for this observation.

81. In a similar study involving blood from four healthy persons and with x-rays doses of 0.05 to 0.5 Gy (4 levels), Stenström [S95] found that the pattern of SA (the involvement of specific chromosomes) varied between the different individuals and that in two cases, irradiation seemed to have a definite influence on this pattern although the chromosomes involved were not the same ones. The changes in the SA tendency were almost opposite to each other.

82. In summary, in spite of the considerable amount of work that has gone into studies on satellite associations, no clear conclusions can be drawn with respect to their relevance in the context of the aetiology of trisomies. Furthermore, the spectrum of chromosome abnormalities in spontaneous abortuses does not support the idea that acrocentric chromosomes are preferentially involved in non-disjunction; the data suggest, rather, that there may be differences in the extent to which the different resultant trisomies (for acrocentric and non-acrocentric chromosomes) survive foetal life.

9. Non-disjunction in the human male studied through direct examination of the germ cell stages

(a) Spermatocytes

83. The 1977 report considered some data bearing on the detection of aneuploidy in human spermatozoa. Briefly, the findings were:

(a) The Y chromosome appears as an intensely fluorescent dot in interphase nuclei after staining with quinacrine dichloride [P2];
(b) With similar staining, the Y chromosome can be seen throughout the male germ cell series [P3] including the spermatozoa [B5, S7].

These findings raised hopes that these F (fluorescent) bodies may be potentially useful for estimating non-disjunction rate of the Y chromosome. In initial studies, it was found that between 1 and 3% of human spermatozoa contained two F bodies [P3, B6] and these frequencies were confirmed in a subsequent study [P4]. As was pointed out in the 1977 report, the assumption that each F body in 2F spermatozoa corresponds to a Y chromosome yields very high estimates of non-disjunction rate for the Y which are inconsistent with the number of XXY individuals (zygotes) found in the liveborn (or abortion) studies.

84. Further work by Beatty [B7, B8, B9] and Sumner and Robinson [S8] has clearly demonstrated that not all Y chromosomes are represented by F bodies and that not all F bodies represent Y chromosomes. In one study [B8], only 83% of the Y chromosomes were represented by F bodies; 7% of the haploid heads and 14% of the diploid heads contained one or more "adventitious bodies" indistinguishable from true F bodies. Indirect DNA estimates of Sumner and Robinson [S8] also lend credence to the idea that a one-to-one correspondence between 2F and YY is unlikely and that many of the
2Fs are not YY's. Furthermore, it is known that single Y chromosomes may have a bifid structure and extra dots in spermatocytes may be explained by single Y chromosomes being bifid (see also [89] for a treatment of the problem).

85. All these lines of evidence strengthen the conclusions reached in the 1977 report namely, that the F bodies may not at present provide a useful means of estimating the rate of Y chromosomal non-disjunction.

86. Notwithstanding these considerations, Kapp [K3] has argued for the use of Y bodies as a tool to monitor Y chromosome non-disjunction in males. His data pertain to a normal male with no known exposure to mutagens, two patients (one who underwent adriamycin treatment for osteogenic sarcoma and who had a prior history of chemo- and radio-therapy and the other who underwent serial x-ray therapy for seminoma), a physician who began fluoroscopy residency, an individual with intestinal amoebiasis who underwent diagnostic irradiation 9 days before starting on a 10-day regimen of Flagyl, a group of 18 men who had experienced occupational exposure to dichloropropane (DBCP) and 50 unexposed controls. In the case of the normal male, the average frequency of "2F" sperm was 1.3% (30 samples over 400 days: range: 0.7 to 2.2%). Likewise, 43 out of 45 controls from the DBCP study had "2F" sperm in the range from 0 to 2%. In individuals undergoing chemical or radiation treatment, the frequency was in general elevated reaching levels of between 3 and 6% in about a month after the beginning of the treatment. In the case of DBCP workers (duration of exposure not specified), 16 of the 18 had frequencies of "2F" sperm over 2% while for 2 of them, the frequency was in the range of 0 to 2%.

(b) Meiotic stages

87. Holm and Rasmussen [H13, H14]. Holm, Rasmussen and von Wettstein [H15] and Rasmussen and Holm [R4, R65] carried out a detailed analysis of the meiotic prophase and metaphase I of human spermatocytes through three-dimensional reconstructions of the meiotic nuclei from electron micrographs of serial sections. The reconstructions comprise 4 leptotene [H13], 4 early-mid zygotene [R4], 10 late zygotene [R4], 21 early pachytene [R4], 22 mid-late pachytene [H13] and 3 metaphase [H14] nuclei and elucidate details of chromosome pairing, chiasma formation and disjunction in man. Of interest in the present context is their finding that suggests that primary non-disjunction can arise in either of two ways. The first is the failure of homologous chromosomes to form a chiasma, their entering the metaphase plate as univalents with the possibility of orientation of the centromeres towards the same spindle pole. The second is premature dissolution of the synaptonemal complex or chromatin chiasma leading to univalents in the metaphase plate with the possibility of their subse-

7 Chromosome pairing and synaptonemal complex formation are intimately related events in meiotic prophase. With the exception of the Drosophila male (and probably other dipteran males as well) which lack synaptonemal complexes, the regular disjunction of homologues appears to be connected with the presence of synaptonemal complexes. In organisms with achiasmate meiosis and/or lacking crossing over, the entire synaptonemal complex complement is retained until the homology chromosomes at anaphase I. In most organisms however, the bulk of the synaptonemal complexes is shed from the bivalents after pachytene leaving only short fragments behind. It is generally believed that these fragments stabilize the cross overs, thereby holding the two homologues of each bivalent together (see [R4, R65] for details and pertinent literature).

10. Detection of chromosome anomalies in human sperm through direct cytological analysis; interspecific cell-fusion studies

88. Rudak et al. [R1] described a technique for a direct analysis of the chromosome constitution of human spermatozoa. In this method, human spermatozoa (in semen samples) are fused with zona-pellucida-free eggs of the golden hamster (Mesocricetus auratus) using the eggs as "reactivating vehicles". To obtain large numbers of eggs, adult female hamsters were induced to superovulate by i.p. injection of 25 IU pregnant mares' serum gonadotrophin on day 1 (the day of ovulation) of the animals' oestrous cycle, followed by an i.p. injection of 25 IU human chorionic gonadotrophin on day 3. The animals were killed 17 h after the second injection, their oviducts dissected out and the cumulus mass containing the ova removed; after suitable washing and clearing of the ova, the zona pellucidae were digested off with 0.1% trypsin at room temperature. Approximately 40–50 eggs were recovered from each superovulated female and eggs from 4 animals were routinely used for each semen sample. The zona-pellucida-free eggs were subsequently transferred to sterile petri dishes containing the medium and mineral oil.

89. To effect sperm penetration, two to three drops of the preincubated sperm suspension were dropped onto a Pasteur pipette onto the surface of the oil in each dish and mixed with the eggs. The dishes were incubated at 37°C in 5% CO2 in air for 3 h. Under the conditions of the experiment, approximately 75% of the eggs which had been incubated with sperm reached the fertilisation stage. Of the fertilised eggs, slightly more than half contained discrete haploid sets of both the hamster and the human metaphase chromosomes. Of the 60 sperm analysed, 31 had a 23,X and 23,Y constitution while 3 were aneuploid (with 22,X, –G; 22,X, –F, and 24,X, +car, +ace, +complement). The frequency of aneuploidy in this sample is therefore 5%. The authors suggest that their technique for the first time permits the preparation and analysis of sperm chromosomes with the same precision as has been achieved for the chromosomes of somatic cells and that "... the way is now open to studying directly the chromosome constitution of a population of sperm ... and to assessing the effects of various natural and experimentally induced phenomena on the chromosomes of mammalian gametes".

11. Cytogenetic studies in offspring of atomic bomb survivors of Hiroshima and Nagasaki: further data

90. Somatic chromosomes of the children of atomic bomb survivors and controls have been examined since 1967 in an attempt to determine whether atomic bomb exposure of parental germ cells may have led to an
increased risk of genetic damage expressed as an increased frequency of children with induced chromosomal abnormalities. The data of Awa [A7] presented in the 1977 report showed that among 2885 children of atomic bomb survivors, a total of 18 individuals (0.62%) with chromosome anomalies were found. In the matched controls (1090 subjects) there were 3 individuals with chromosome anomalies (0.28%). There was a suggestive, but statistically non-significant increase in the frequency of sex-chromosomal anomalies in the children of exposed parents (0.31 versus 0.09%). Finally, the total frequency of chromosomally abnormal children of atomic bomb survivors was in the same range as that which has been reported in several newborn surveys (see Table 2).

91. Awa et al. [A8] have now updated this information (see also Ref. S131). Chromosome analysis has been completed for a total of 10,820 children comprising 5058 controls (children of distally exposed parents) and 5762 children of proximally exposed parents with an estimated conjoint gonadal exposure of 0.87 Sv. The results are that in the controls, 25 (0.49%) were identified as having an abnormal chromosome constitution (11 with balanced autosomal rearrangements, 1 unbalanced rearrangement, and 13 sex-chromosomal aneuploids). Among the children of the exposed parents, there were 30 chromosomally abnormal individuals (0.52%) the break-down being, 11 balanced autosomal rearrangements, 3 unbalanced autosomal rearrangements, and 16 with sex-chromosomal anomalies. The present data, based on more than twice the sample size, suggest again no significant difference in the frequencies between controls and the children of exposed parents.

12. New chromosomal abnormalities and birth defects

92. The 1966 report of the Committee [U7] dealt with some of the well-known chromosomal anomalies in humans such as trisomy 21, trisomy 18, trisomy 13, Cri-du-chat syndrome (deletion of the short arm of chromosome 5), partial trisomy for chromosome 21, deletions particularly involving the short or the long arms of chromosome 18, D/D and D/G translocations and the sex-chromosomal anomalies. In the 1972 report [U8], further information on chromosomal anomalies particularly on reciprocal and Robertsonian translocations was presented including methods of ascertainment (through a balanced or an unbalanced proband) and their relationship to the transmission of the translocations.

93. The application of banding techniques (reviewed by Dutrillaux [D5] and Dutrillaux and Lejeune [D2]) to study human chromosomes has revolutionized the field of human cytogenetics and has led to major advances. Among these, the following may be listed:

(a) The confirmation of well-established chromosomal syndromes (e.g. +8, +13, +18, +21, 4p-, 5p-, 18p-, 18q-, etc.);

(b) A precise identification of previously suspected trisomies (e.g., trisomy 8 and monosomies such as those involving chromosomes 21 and 22);

(c) A sub-division of classical chromosomal syndromes according to the chromosome segments involved (e.g., partial deletions involving the proximal one-third to one-half and the distal one-third to two-thirds of the long arm of chromosome 13; partial deletion of chromosome 18: 18p- and 18q- syndromes; the 21q- syndrome);

(d) The delineation of new chromosomal syndromes involving practically every segment of the different chromosomes of the human complement;

(e) A more precise definition of known chromosomal anomalies (e.g., the identification that the relevant chromosome segment in the Cri-du-chat syndrome (5p-) is in the mid-position of 5p15 band);

(f) The discovery of fragile sites in certain chromosomes;

(g) The demonstration of extensive chromosomal heteromorphisms in humans and their applications;

(h) The demonstration of an association between (i) certain genetic diseases and specific chromosomal anomalies (e.g., retinoblastoma: specific loss of band 13q14) and (ii) certain neoplasias and chromosomal aberrations (e.g., chronic myeloid leukaemia (CML) and the Philadelphia (Ph1) chromosome);

(i) Progress in gene mapping;

(j) Evolutionary studies in primates.

94. Most of the above items have been adequately covered in several recent reviews [G2], [G3], [S16], [N9], [N10], [R8], [R9], [V4], [P8], [Y2], [F5], [W6], [H11] & [K5], [S23], [C19], [M21], [L8], [B12], [D3] and [D4]. In what follows therefore, only certain important features will be summarized with particular reference to partial monosomies and trisomies and fragile sites.

(a) Partial monosomies and trisomies

95. As mentioned earlier, new chromosomal defects, particularly deletions and duplications involving almost every chromosome of the human complement have been discovered during recent years (see Table 2 in [S23] and Table 1 and Figure 1 in [L7]). The rate at which information is accumulating justifies the conclusion that the incidence figures for chromosomal anomalies summarized in Table 2 may need upward revision. Most of the currently available information on these partial deletions and duplications, however, is in the nature of case reports. For some such as Cri-du-chat syndrome, the recent review of Niemeyer [N9] shows that the incidence rate may be of the order of 1 in 45 000 and among mentally retarded individuals the rate may be of the order of 1.5 per 1000. There was a significant excess of females with this syndrome.

96. Several chromosome deletions with distinctive features have counterparts in single gene defects which are inherited as autosomal dominants. This seems to be the case with retinoblastoma which is associated with an interstitial deletion of the long arm of chromosome 13, specifically, band 13q14 [Y3]. Lewandowski and Yunis [L7] have cited several other possible instances of this kind.

97. In general, partial trisomies seem to be more frequent than partial monosomies, presumably because the former are less deleterious. Roughly two-thirds of the reported cases (83 out of 129) summarized by Sanchez and Yunis [S23] are partial trisomies and the rest, monosomies. From their detailed analysis of 95 balanced reciprocal translocations (the break-points, the chromosomes and the chromosomal segments

8 It is worth mentioning here that what have hitherto been considered cases of trisomy-22 are really translocations involving chromosomes 11 and 22 with 11q being the predominant trisomic segment [D6].
involved, the type of segregation observed in the families ascertained, etc.) Auriya et al. [A3] reached a similar conclusion, that trisomies are relatively better tolerated than monosomies. Their results show that in the case of 1:3 segregation (leading to monosomies), there is an average imbalance of 1.68 U° whereas in the case of 3:1 segregations (leading to trisomies) the average imbalance is 4.22 U. Furthermore, with 2:2 segregations, the segment in triplicate is generally longer than the monosomic segment (TM10 translocations: 3.85 U long trisomy for a 0.47 U long monosomy; non-TM translocations: 2.63 U trisomy for 1 U long monosomy). The authors point out, however, that there are some exceptions and that other causes in addition to length may therefore be involved in influencing the severity of chromosome disorders.

98. Another finding of interest that emerges from the work of Auriya et al. [A3] is that there is an excess of breakpoints for chromosome arms 4p, 9p, 10q, 21q and 22q and a deficiency of breakpoints in 1p, 2p and 6p.11 With the exception of chromosome 22, all the others (4, 9, 10 and 21) are implicated in the aetiologies of relatively frequent chromosomal disorders (for reviews see [G2, R8, R9, Y2]). As one plausible explanation, the authors suggest that monosomies and trisomies of these segments may be relatively well tolerated and that the translocations involving these chromosomes are therefore more readily ascertained, especially in children carrying unbalanced karyotypes.

99. Although trisomies and monosomies may involve any chromosome, the amount of excess or deficient material does not generally exceed 5% of the total genome (see table 2 in Sanchez and Yunis [S23]). A concept that seems to be gaining increasing currency in recent years is that duplications or deletions of late replicating regions are less harmful than those involving earlier replicating regions. Positive Q- and G-bands are known to represent late replicating regions [G4, C17, C16, D5] and the chromosomal segments generally involved in the chromosomal syndromes are particularly rich in Q- or G-positive bands.

100. In contrast to "classical" chromosomal syndromes (e.g., trisomy 21, 18, 13, etc.) which are relatively well-defined entities (and which can be recognized on clinical grounds alone) some partial deletions and duplications are presently difficult to diagnose either because of the absence of a specific relationship between certain phenotypes and chromosomal segments or because of overlapping phenotypic effects. In addition, the chromosomal segments involved may vary from patient to patient and consequently, the phenotype may show considerable variation. For instance, offspring from carriers of balanced reciprocal translocations may receive derivative chromosomes. This leads not only to partial monosomy or trisomy, but usually to a combination of both which can be translated into various mixtures of two given syndromes.

(b) Heritable fragile sites

101. A class of chromosomal entities that currently engages the attention of human cytogeneticists is constit-

9 U is the unit length of the autosome (total length: 280 units).
10 TM: breakpoints in the telomeric and median regions.
11 p and q refer, respectively, to the short and long arm of the chromosome.

102. Heritable fragile sites have been found on a number of metacentric or sub-metacentric chromosomes (2, 10, 11, 16, 20) and at least one is known on the X; however, they have so far not been found in any of the five acrocentrics (13-15, 21, 22) or the Y. They are never seen in 100% of the cells examined.

103. Lub's [L43] examined DNA replication by autoradiography in a female with a site Xq27 (or 28) and found that the X with the fragile site did not appear to be selectively inactivated. Fraccaro et al. [F23] similarly studied a site at 2q1 and found that in most cells there was no detectable asynchrony in DNA synthesis between homologues. Sutherland and Leonard [S97] showed that the chromosomal gaps associated with the fragile sites at 2q11, 10q23, 11q23, 12p11, Xq27 (or 28) do not stain with silver nitrate as the nucleolar regions of the acrocentric chromosomes do.

104. Sutherland [S96] demonstrated that the expression of fragile sites in metaphase chromosomes obtained from lymphocyte cultures occurred only when culture medium 199 (which is relatively deficient in folic acid) was used, compared to several other media i.e., their expression depends on the composition of the culture medium. This was true for sites 2q11, 10q23, 11q13, 16q124, 20p11, Xq27 (or 28) but not for the site 16q22. The expression of these sites (except that at 16q22) was inhibited by folic acid, thymidine, formic acid and probably BuDR. The inhibition, however, could be reversed by a folic acid antagonist, methotrexate. In addition, there was a correlation between the frequency of the sites and the pH of the medium for the sites at 2q, 10q and Xq. It was therefore postulated that there may be at least three different biochemical classes of fragile sites, as judged by their response to pH, folic acid and methotrexate. In a recent report, Schmid et al. [S98] showed that the presence of the antibiotic distamycin A in the medium can reveal the fragile site at 16q22 although there were interfamilial differences in its appearance.

105. In a recent study, Glover [G38] confirmed the findings of Sutherland, namely that folic acid and thymidine inhibited the expression of the fragile site on the X-chromosome and further showed that the inhibiting effect of folic acid, but not that of thymidine, can be negated by the addition of 5-fluorodeoxyuridine (FUDR) to the culture medium. He suggests that this observation can be explained by the fact that FUDR is intra-cellularly converted by thymidine kinase to 5-fluorodeoxyuridine monophosphate (FdUMP) which in turn, is a potent inhibitor of thymidylate synthetase. In the absence of exogenous thymidine, the pool of deoxythymidine monophosphate (dTMP) is depleted.
thus arresting DNA synthesis. Exogenous thymidine bypasses this block by conversion to dTMP catalysed by thymidine kinase in the "salvage pathway". In other words, the observations are consistent with the hypothesis that the fragile X is expressed by limiting the dTMP pool and, thus, the deoxythymidine triphosphate (dTTP) pool available for DNA synthesis. The data do not support the notion that there is a deficiency in thymidylate synthetase in individuals with the fragile X since inhibition of this enzyme does not result in fragile X expression in control males.

106. In 1980, Sutherland et al. [599] reported finding a new fragile site at 1q25. This site differed from others in that it required BUDR (at a concentration of 5–10 mg/litre) in the medium for maximum expression frequency, and was not inhibited by folic acid concentrations of up to 20 mg/litre or by pH. High concentrations of thymidine however, tended to inhibit expression. The finding of BUDR-requiring fragile site at 1q25 has also been reported by Scheres and Hustinx [S100].

107. Most of the fragile sites discussed above have been found either in the course of population cytogenetic studies or in individuals suspected to be chromosomally abnormal. Sutherland [S101] points out that with autosomal fragile sites, there is no association with abnormal phenotypes and that the detection in the instances mentioned earlier is probably a reflection of the material analysed. However, the fragile site at Xq27 seems to be associated with, and may even be the cause of, the form of mental retardation with macro-orchidism [e.g., G37, H52, H53, H54, J17, S99, S101, S102, S103].

108. In a study of 21 mentally retarded males with macro-orchidism, 13 obligate carrier females and 26 potential carrier females, Sutherland [S102] found that in the males, the frequency of cells showing the fragile site Xq27 ranged from 4 to 56%. Among the obligate carriers, only 5 showed any evidence of the fragile site and all of them were younger than 35 years; in 7 of the remaining 8 and who were older than 35, the fragile site could not be demonstrated. In the last case, the fragile site was readily demonstrated at age 30, but not at age 32. In only 9 of the potential carriers could the fragile site be shown to occur. The ages of these individuals ranged from 2.5 to 21 years. After reviewing these and other data published in the literature, Sutherland [S102] concluded that the diagnosis of X-linked mental retardation with macro-orchidism remains difficult; that not all the retarded males with the fragile site have macro-orchidism; that the fragile site can often be demonstrated in only a small proportion of metaphases from some retarded males even when the diagnosis is virtually certain on clinical grounds and from a study of the family history including other affected relatives; and that the detection of the fragile site in females is still inadequate despite the methods developed to manipulate the culture medium.

109. A recent report by Daker et al. [D19] has raised the possibility that an X chromosome with a fragile site typical of X-linked mental retardation can occur in normal individuals; the Xq28 was found in a male patient (referred to for cytogenetic examination for other reasons) and in his brother, both of whom appeared to be of normal intelligence. The authors point out that "... although one case can hardly undermine the importance of fra(X) in the diagnosis of X-linked mental retardation, nevertheless, the knowledge that this fragile site may occur in individuals of normal intelligence will make genetic counsellors feel a little uneasy, especially if prenatal diagnosis of the fra(X) becomes a reality ... this case therefore clearly indicates the need for more information about fragile sites, especially in the 'normal' population".

13. Summary and conclusions

110. The available results of 10 cytogenetic surveys of neonates (carried out in different parts of the world) show that 0.63% of the babies (424/67 014) are chromosomally abnormal; 158 children (about one-third) carried sex-chromosomal anomalies, 93 children (about one-quarter) numerical chromosome anomalies, 134 children (about one-third) balanced structural anomalies of the autosomes and the remainder (37/424) unbalanced structural anomalies. The incidence of sex-chromosomal anomalies alone is about 3 per 1000 male births and 1.5 per 1000 female births.

111. Of the autosomal numerical anomalies (about 1.4 per 1000 births), trisomy 21 constitutes the predominant group. Among the balanced autosomal structural anomalies (about 2 per 1000 births) reciprocal translocations and Robertsonian translocations are about equally frequent. Among the latter, those involving two D group chromosomes are more common (48 out of the 60) than those involving D and G group chromosomes.

112. About one-half of all the abnormalities detected at birth may be deemed to be clinically significant (about 3.3 per 1000 births). These abnormalities include all the non-mosaic XXY and 45.X cases, XXX, XYY and XX (male) conditions, all autosomal trisomies and all unbalanced structural rearrangements reported to have been associated with congenital malformations at birth. There are several reasons why this frequency is likely to be an underestimate.

113. The data on the incidence of chromosomal anomalies in newborn infants and other results bearing on whether these are familial or newly arisen, have been used to arrive at estimates of mutation rates. For those abnormalities resulting in livebirths, the estimated rates are the following: numerical errors of autosomes, 6.9 10−4/gamete/generation; sex-chromosomal numerical errors, 8.3 10−4/gamete/generation; balanced Robertsonian translocations, 0.40 10−4/gamete/generation; balanced reciprocal translocations, 1.3 10−4/gamete/generation; unbalanced Robertsonian translocations, 0.23 10−4/gamete/generation; and unbalanced non-Robertsonian structural rearrangements, 0.58 10−4/gamete/generation.

114. In perinatal deaths, the frequency of chromosomal anomalies has been estimated to be of the order of 5 to 6%. Among the anomalies found, trisomies predominate.

115. Further data on the incidence of chromosomal anomalies in spontaneous abortuses (published subsequent to the 1977 report) confirm and extend the conclusions reached in the 1977 report: about 50% of spontaneous abortuses are chromosomally abnormal and trisomies as a group account for over one-half of all chromosomal anomalies recorded in the abortuses. The trisomies have been found to show a maternal-age dependence and the latter is pronounced for those involving the small chromosomes, both aero-centric and non-aero-centric. Trisomy 16 is different from the other
trisomies in that there seems to be no demonstrable maternal-age dependence.

116. Insights into the origin of the extra chromosomes in some trisomies have been gained through the use of chromosomal heteromorphisms as cytogenetic markers. The conclusions that may be drawn from the data are that in the abortus material, for trisomy 16, non-disjunction can occur at any one of the meiotic divisions in either sex, with meiotic I error in the female predominating. For others (trisomy 13, 14, 15, 21 and 22), non-disjunction seems to occur almost exclusively at division I in the female. Trisomy 21 in livebirths seems to arise primarily (in over 70–80% of the cases) as a result of non-disjunction in the mother (division I).

117. There are now extensive data demonstrating that in the case of Down's syndrome, there is an increase in the frequency with increasing maternal age. There are also some reports suggesting that increased paternal age may also play a role.

118. The question of whether there is an increase in the frequency of Down's syndrome children among the progeny of irradiated mothers is not yet settled: the several prospective and retrospective epidemiological studies carried out thus far to specifically investigate the problem have not provided unequivocal evidence in this regard.

119. Studies aimed at testing whether or not satellite associations between acrocentric chromosomes seen in metaphase preparations of lymphocytes may be related to the aetiology of trisomies for these chromosomes, have not provided evidence for such a relationship. Irradiation was found to cause changes in the pattern of satellite associations, but the significance of such changes is not amenable to any satisfactory interpretation.

120. The use of banding techniques to study human chromosomes has led to a number of major advances among which are the discovery of new chromosomal defects particularly partial monosomies and trisomies, and heritable fragile sites. In general, partial trisomies seem to be more frequent than partial monosomies. Heritable fragile sites have been found on a number of metacentric or sub-metacentric chromosomes and at least one is known on the X. Their expression depends on the composition of the tissue culture medium. The relationship between the heritable fragile sites and birth defects is not yet clearly established. However, the fragile site on the X (Xq27 or 28) is usually associated with the form of mental retardation with macroorchidism in males; there is however one recent report which raises the possibility that an X chromosome with a fragile site typical of X-linked mental retardation can occur in normal individuals.

D. GENES, CHROMOSOMES AND CANCER

1. Monogenic disorders and neoplasia

(a) Introduction

121. Many single gene traits predispose to, or are complicated by, neoplasia [G5, J6, K6, L9, M22, M23, S24]. From the fourth edition of McKusick's [M17] Catalogue,Mulvihill [M23] could extract over 200 conditions with neoplastic tendencies. i.e., with benign or malignant neoplasia or tumour as the sole feature, a frequent concomitant or just a rare complication (see table 1 in [M23]). Among these 200 conditions, about one-half are autosomal dominant, one-third autosomal recessive and one-sixth X-linked; for some, evidence for Mendelian behaviour is clear-cut while for others such evidence is inconclusive. Some of the traits, because of their rarity, are represented by only single case reports. Notwithstanding these limitations, Mulvihill's analysis shows that a substantial number of all known single gene traits in humans can be manifested as neoplasias; that a large number of gene loci might be involved in cancer susceptibility, and by inference, the number of "normal" genes contributing to resistance to neoplasia is large, and nearly all bodily systems and histological types of tumours are represented including the commonest malignancies of skin, breast, colon and lung.

122. The better known of these traits include xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Fanconi's anaemia (FA), Bloom's syndrome (BS) and retinoblastoma. All these except retinoblastoma are inherited as autosomal recessives. In AT, FA and BS, spontaneous breakage of the chromosomes occurs in peripheral blood lymphocytes and in cultured fibroblasts. On this basis, these syndromes are correctly referred to as "chromosome instability syndromes". In recent years, other disorders such as porokeratosis of Mibelli (autosomal dominant), nevoid basal cell carcinoma (autosomal dominant), incontinentia pigmenti (X-linked) and scleroderma (multifactorial) which may fall under chromosome instability syndromes have come to light. German [G35, G36], Hecht and McCaw [H21] and Paterson [P33] have reviewed the main findings. A workshop [W24] recently held in England was entirely devoted to progress in research with AT. In what follows, some salient features of XP, AT, FA, BS and retinoblastoma will be summarized with special reference to neoplasia.

(b) Xeroderma pigmentosum (XP)

123. The main feature of XP is a marked sensitivity of the skin to sunlight-induced damage, manifested as sunburns, freckling, hyperpigmentation and keratoses, eventually leading to multiple skin carcinomas and melanomas which are the final cause of death usually before the age of 30 [R10]. The disease has now been successfully diagnosed in utero [R11]. Clinical heterogeneity of XP is manifest by a subgroup characterized by mental retardation and neurological abnormalities (De Sanctis-Cacchione syndrome). The basis for predisposition to cancer is a metabolic abnormality in the repair of UV-induced damage to DNA and is considered in a later section.

124. XP patients do not show increased numbers of chromosomal aberrations, although there are isolated reports of a pseudodiploid clone [G6] and of an increase in abnormalities in fibroblasts from XP individuals at late but not at early passage in culture [H22]. The levels of sister chromatid exchanges (SCEs) appear normal [B13, K7, W7].

125. XP individuals have been found in all geographic groups although their frequency seems to vary in different populations. For the populations from North America and Europe, the frequency has been estimated at about 1 in 250 000 [R10]; in Japan this has been estimated to be higher, being 1 in 40 000 to
100,000 [T27]. While homozygotes are very sensitive to sunlight-induced cancer (and die due to this), the situation in heterozygotes is not yet clear [S25, T27].

(c) Ataxia telangiectasia (AT)

126. The major findings associated with AT are progressive cerebellar ataxia, conjunctival and cutaneous telangiectasia, frequent sinopulmonary infections with abnormal immunity (not in all patients), a generally hypoplastic lymphoid system and a predisposition to cancer (see [B14, H23]). Most of the cancers reported in AT patients involve the lympho-reticular system; less frequently, epithelial tumours and leukaemia have also been reported [H23]. Death often occurs before the age of 20 either from sinopulmonary infections or from malignancies so that it is difficult to estimate the absolute risk of malignancy (AT patients with cancer are more likely to be reported). There is however no doubt that the risk of malignancy, at least of the reticuloendothelial system, is greatly increased in AT [H21, S25]. An extensive comparison of the many clinical aspects of XP and AT has been made by Kraemer [K8].

127. In many, but not all, AT patients, increased chromosome breakage is evident in lymphocytes, and less strikingly, in fibroblasts [G16, H21, H23, H29, L11]. The SCE frequencies are in the normal range, although there is some variation between different patients [G7, B13, H24]. The non-random chromosomal changes that have been recorded in AT individuals are discussed in the next section.

128. Homozygotes for the AT gene may occur as often as 1 in 40,000 births [S25, S26]. From this, the frequency of heterozygotes (assuming Hardy-Weinberg equilibrium) can be estimated to be about 1% of the population (the latter calculation assumes that AT is genetically homogeneous which it probably is not; see, for instance, [H21, P37]). One report about AT patients (cited in [S25]) noted a number of cancers in family histories in their clinical records. The relatives had no signs of AT and many may have been heterozygous for the AT gene. Swift et al. [S27] found that for blood relatives of AT individuals (27 families) there was an increase in deaths from all types of malignancy, primarily in younger persons (<45 years). Below age 45, there were 15 deaths due to malignancy (5.2 expected) and below age 75, there were 59 deaths (42.6 expected). There were actually fewer deaths from cancer than expected in persons dying after 75 years of age. Furthermore, the ratio of observed to expected deaths from malignant neoplasms increased with an increase in the probability of heterozygosity for the AT gene [S25, S27].

129. The estimates of risks for heterozygotes dying of cancer (relative to normal controls) varies by factors of between 2 and 10 depending on how the data are analysed. For instance, the relative risk factor is 2 when death due to all malignancies is considered; it is 5.5 when cancers occurring below age 45 years alone are considered and it rises to 10 when ovarian tumours in women of <55 years are taken into consideration. These values of relative risks multiplied by the estimated heterozygote frequency give a value which is a useful measure of the proportion of all cancer patients who carry the AT gene. For instance, over 5% of all persons dying before age 45 from any malignancy may carry the AT gene [S25].

(d) Fanconi’s anaemia (FA)

130. Fanconi’s anaemia, also termed Fanconi’s pancytopenia or Fanconi’s constitutional infantile pancytopenia is a chromosome instability syndrome associated with progressive marrow failure [F6]. The clinical features include progressive underdeveloped type of red cells, white cells and platelets leading to anaemia, leukopenia and thrombocytopenia, hypoplasia or aplasia of the radius and thumb, growth retardation and brownish pigmentation of the skin [B15, G6, H21]. Other skeletal malformations and anomalies of the heart and kidney also occur. Death occurs mainly in the early years, but those who survive longer have an increased risk of acute leukemia, especially myelomonocytic. Affected patients are also at a greater risk for developing squamous cell carcinoma of mucocutaneous junctions, such as around the mouth and anus and for hepatic adenoma, especially following prolonged androgen therapy for their pancytopenia [H21].

131. FA patients show increased chromosome breakage and rearrangement, most evident in fresh bone marrow preparations and in lymphocytes cultured for short periods of time such as two to three days; these occur also in fibroblasts cultured for longer periods. The breaks are usually of the chromatid type [S38, S39, S40, W12] and the chromatid interchanges observed (unlike in Bloom’s syndrome patients) are mainly between non-homologous sites. The levels of sister chromatid exchanges are normal [H25, K7, L16].

132. Dutrillaux et al. [D11] made a study on the localization of chromatid breaks in lymphocytes of three FA patients using three consecutive stains (Giemsa, Q and R banding). The breakpoints were almost exclusively located in the interbands between R and Q bands, the only true exceptions being the secondary constrictions. The breaks seemed over-represented in the larger autosomes 1–13 (excepting 4, 8 and 10) and under-represented in the smaller ones (excepting chromosome 17); the sex-chromosomes were only rarely affected. There was a clear over-representation of breaks at three sites: 1q12, 9q12 and the interband between 6p21 and 6p22. The remaining breaks were randomly distributed.

133. The incidence at birth of FA is about 1 in 350,000 for the North American population [S25, S31] and may be higher (1 in 70,000) in mid-Europe [W8]. For the former population, a heterozygote frequency of about 0.33% can be estimated. In eight families of patients, 25 deaths from malignancy (among 102 deaths) were found, a frequency which is significantly higher than the 15.0 expected [S25, S31, S32]. This finding of increased cancer risk for FA heterozygotes does not appear to have been substantiated in some other families studied by Swift.

(e) Bloom’s syndrome (BS)

134. Three main clinical features of BS are: severe growth retardation, a telangiectatic erythema on exposed areas and sun-sensitivity [B16, G6, G9, G10, H21]. Many patients have serious respiratory and intestinal infections and the immune system is impaired. The risk of cancer is increased in BS patients. Primary cancer develops in approximately 1 out of 6 patients. About half of the cancers are leukaemias of the non-lymphocytic type [H21, G11]. Roughly half of the cases recorded in the literature have been Ashkenazi Jews. A
founder effect is evident and the affected Ashkenazi Jews have been traced to a small area at the border of Poland and the Ukrainian Soviet Socialist Republic. It is not known whether relatives of patients have an increased risk of malignancy.

135. The cytogenetic hallmark of BS is the symmetrical quadriradiolar figure, which is rarely seen in other chromosome instability syndromes. Lymphocytes and fibroblasts show an increase in quadriradiolar figures. The quadriradiolar figures involve homologous chromosomes [G10, G12, S30]. This is so characteristic of the disease that, according to German [G10], BS is not diagnosed unless these quadriradiolar figures are found. The exchanges occur preferentially near the centromeres [G6, G13, S33] and are distributed non-randomly along the chromosomes. In the bone marrow such interchanges have not been found though other aberrations have been detected in some patients [G6, S33, S34].

136. The cells from BS patients contain highly elevated frequencies of sister chromatid exchanges (approximately 90/cell; leucocytes, fibroblasts and bone marrow [B13, C20, G14, S34, S35]). The only known defect in cultured cells is a reduced rate of DNA chain elongation [H55]. However, this impairment does not alter synthesis post pyrimidine dimers in template DNA i.e., post-replication repair is normal [G22].

137. Some reports suggest that the characteristically high level of spontaneous SCEs in BS cells can be reduced. In studies involving co-cultivation of BS and CHO cells, van Buul et al. [B21] showed that the SCE frequencies in BS cells can be reduced by about 20%; the effect was observed only when cell-to-cell contact was present with CHO cells without any effect on the SCE frequencies in CHO cells. Bryant et al. [B22] reported more dramatic results: in euploid cell hybridization studies, they were able to demonstrate that the high SCE frequencies in BS cells could be reduced to normal levels thus resulting in a complete "correction" of the mutant phenotype.

Retinoblastoma

138. Retinoblastoma, an embryoma of the precursors of the rod and cone cells in the retina, is a malignant eye tumour of children [B17, D10]. Mortality is associated with direct extension of the tumour into the cranial cavity to involve the brain and leptomeninges. The incidence figures for this condition reported in the literature (reviewed in ref. [V3]) range from about 1 in 34 000 (Holland, 1927-1929) to about 1 in 10 000 (two African populations; recent figures). Vogel [V5] considered that the most reliable estimates range between 1 in 50 000 and 1 in 15 000 and are based on studies which involved a more complete ascertainment.

139. Retinoblastoma is often considered a classical example of a dominantly inherited tumour. As is now well-known, this is not true of all retino-blastomas. Analysis of the data indicates that about 60% of all retino-blastomas are unilateral and non-hereditary, 15% are unilateral and hereditary and 25% are bilateral and hereditary. In hereditary cases, the tumours tend to appear a year earlier than in non-hereditary cases [K9]. For hereditary cases, the penetrance is of the order of 90 to 95% [K10, V5]. Patients with bilateral, and possibly in general with hereditary, retinoblastoma run an increased risk of becoming afflicted with other tumour diseases, such as bone sarcomas in later life [F24, F25].

140. The idea that retinoblastoma is a direct effect of an autosomal dominant gene has been challenged [Z7]. Knudson [K10, K11] proposed a two-mutation model for both the hereditary and non-hereditary retinoblastoma, i.e., the first event a germinal mutation that makes all of the cells susceptible and the second event a somatic mutation that transforms this mutant cell into a tumour cell. Vogel [V5] has suggested that in the non-hereditary variant, a single mutational step, possibly a small chromosome deletion, may be enough to produce a tumour.

141. The possibility that at least in a minority of retinoblastoma cases, there may be an association with a partial deletion of the long arm of a D group chromosome has long been surmised [L10, T6, W11]. Orty et al. [O5] used the banding techniques to the chromosomes of a patient with retinoblastoma and a deletion of a D group chromosome and identified the chromosome as number 13. This finding has been confirmed in several cases [O6, P13]. Six of the ten cases with an interstitial deletion of chromosome 13 reviewed by Niebuhr [N10] had bilateral tumours and one with bilateral tumours had a ring chromosome.

142. Cytogenetic evidence suggests that the locus for retinoblastoma is on the long arm of chromosome 13 (the proximal part of 13q21 or the adjacent 13q14 region [L7]). Most recently, Yunis and Ramsey [Y3] have refined the localization to a portion of band q14 of chromosome 13.

143. Caetzel et al. [C59] examined lymphocyte chromosomes from 12 of the 43 cases included in their survey of Hungarian patients [C60]. These cases were selected solely for technical feasibility of examination. The breakdown of the selected cases was seven sporadic unilateral cases that had been treated with surgery only, two sporadic unilateral cases, two sporadic bilateral cases treated with surgery and x-irradiation and one familial unilateral case treated surgically and with x-irradiation. It was found that there was a significantly higher number of aneuploid cells and cells carrying structural chromosome anomalies such as chromatid and iso-chromatid breaks and stable chromosome-type aberrations. The increase was found not only in the x-irradiated cases, but also in the seven unirradiated ones (the latter sporadic and unilateral). More recent studies by Knight et al. [K33] on twelve patients with matched controls failed to show any increase in chromosome instability in lymphocyte cultures of retinoblastoma patients.

144. There is at least one report [T28] of increased sister chromatid exchanges in fibroblasts from a child with del(13) retinoblastoma. The skin biopsy was performed before the clinical onset of the tumour. The observed frequency of 19.7/cell was significantly higher relative to that in a normal control.

Aniridia-Wilms' tumour-urogenital abnormalities association

145. Aniridia (absence or defect of the iris; specifically congenital hypoplasia of iris) is usually bilateral and is transmitted as an autosomal dominant trait; it affects about 1 in 50 000 of the general population [M57, M58]. About 30% of the cases are sporadic and
are presumed to represent new germinal mutations [B17]. Wilms' tumour is an embryo of the kidney derived from metanephric blastema. The incidence rate has been estimated to be of the order of 1 in 10 000 live births or 6–7 per year per million children under age 15 [Y7]. Wilms' tumour is discovered most often between ages 3 and 4 at which time it is extremely malignant [G39]. These tumours are bilateral in 5 to 10% of the cases, and the bilateral tumours may develop simultaneously or sequentially [B17].

146. Familial Wilms' tumour occurring in siblings has been reported by a number of investigators [B63, B64, K34, M59]. Knudson and Strong [K34] reviewed and summarized data on 58 familial cases. They concluded that bilateral tumours are more likely to be familial, that familial tumours result from two mutations, one germinal and one somatic and that sporadic tumours result from two somatic mutations.

147. It has been found that the presence of aniridia somehow renders the affected child prone to the development of Wilms' tumour [B17, M60]. Aniridia is present in 1 out of 80 Wilms' tumour cases. The sporadic cases seem more at risk, as about a third of these develop Wilms' tumour. The risk of developing Wilms' tumour seems highest when sporadic aniridia is accompanied by genito-urethral tract malformations and mental retardation. Approximately 6% of patients with Wilms' tumour exhibit upper urinary tract anomalies [J18, K35].

148. Since the original report that the aniridia-Wilms' tumour association with mental retardation and genito-urethral abnormalities in males is caused, at the cytological level, by an interstitial deletion of the short arm of chromosome 11 [F26, R66], there have been several confirmatory observations [A26, F27, Y8]. Francke et al. [F27] concluded that a specific deletion of 11p13 appears to cause aniridia with a 1 in 3 risk for the development of Wilms' tumour and an even greater risk for mental retardation. It is interesting to note that the gene coding for catalase has been recently mapped, on the basis of enzymatic studies, to the same region of chromosome 11 (band 11p13) [J19]. Junien et al. [J19] have pointed out that “from a practical standpoint, an assay of catalase activity would thus become a useful complementary test in patients with aniridia appearing to be new mutations. A low catalase activity would then demand surveillance of the kidneys and gonads, even in the absence of a visible chromosome deletion”.

2. Specific chromosomal defects in cancer

149. It is quite common to find that cancerous cells have a highly aberrant chromosome number, but this aneuploidy probably results from the rapid uncontrolled mitotic activity of such cells, rather than being the cause of it. Banding techniques have permitted to gain some insights regarding the possible role of some chromosomes (or chromosomal changes) in the origin of some neoplasias. More data, however, would be needed for a precise definition of the relationships between specific chromosomal changes and cancer and for an understanding of the mechanisms involved. Mietelman and Levan [M24], Sanchez and Yunis [S23], Harnden [H26], Rowley [R12] and Hecht [H20] have summarized most of the available information in this area.

(a) Chronic myeloid leukaemia and the Philadelphia (Ph1) chromosome

150. In 1960, Nowell and Hungerford [N11] reported the first consistent chromosomal abnormality in a human cancer when they described the abnormally small G group chromosome that they observed in leukemic cells from patients with chronic myeloid leukaemia (CML). This chromosome, which appeared to have lost about half of its long arm, was called the Philadelphia (Ph1) chromosome from the city of its discovery. A number of laboratories subsequently reported that 100% of patients with CML showed the Ph1 chromosome in their bone marrow cells. Others reported that up to 30% of patients with CML were Ph1 negative. In a number of the early cases, only peripheral blood was studied and this might explain why some of the patients were classified as Ph1 negative (see Rowley [R12] for a review and citation of the early references).

151. By using banding techniques, Caperson et al. [C21] and O’Riordan et al. [O4] independently reported that the Ph1 chromosome was no. 22 and that it should be identified as 22q−. The question regarding the nature of the Ph1 chromosome was answered in 1973 when Rowley [R13] reported that the Ph1 chromosome represented a translocation; rather than a deletion as many investigators had previously assumed. The first report presented data on 9 Ph1 positive patients; all of them had additional dually fluorescent chromosomal material at the end of the long arm of one no. 9 (9q+). It was therefore proposed that the abnormality in CML was an apparently balanced translocation (9;22)(q34;q11). Subsequent measurements of the affected pairs (9 and 22) showed that the amount of DNA added to number 9 is equal to that missing from the Ph1 [M25].

152. The original report on the translocation, and a number of reports confirming the observation, noted that the translocation occurred only between number 9 and number 22 (see for instance [D8, P9, P10, P11, W9, W10]). However, subsequently, translocations between number 22 and other chromosomes (numbers 2, 13, 16, 17, 19 or 21) were also reported [e.g., F6, H27, H28, M26, R14].

153. When patients with CML enter the terminal acute phase, about 30% appear to retain the 46,Ph1-positive cell line unchanged, whereas 70% show changes. Among these 70%, chromosomal changes also occur on the Ph1-positive cell line in 70% of patients (see table 2 in Rowley [R12]). A change in the karyotype is a grave sign and, with rare exceptions, heralds the acute blast phase.

154. In a workshop [D9] which was organized to review the clinical and cytogenetic data in CML and acute non-lymphocytic leukaemia (ANLL), data on 223 patients with Ph1-positive CML were compiled and analysed. The prerequisites for inclusion of patients in the series were that bone marrow mitoses had been studied by banding and that sufficient clinical data were available. Of the 223 patients, 122 were studied in the chronic phase, 59 in both the chronic and acute phases, 37 (who were known to have CML) only in the blast phase and 5 (with no prior history of CML) in the blast phase.

155. The major findings were that the translocation between chromosomes 9 and 22 was found in 92% (205) of all patients; that of the remainder, 92% had a two-way translocation involving chromosome 22 and another
chromosome, and 9 had three- or four-way translocations, all of which involved both chromosomes 9 and 22 and some other chromosome(s) (see also [P34]); that one patient with a 22q− lacked an obvious translocation; that fewer than 10% of the patients in the chronic phase had other karyotypic abnormalities. In contrast, at least 75% of those in the acute phase showed changes in their karyotype. In some instances, such changes preceded the onset of clinically apparent blast crisis by up to 18 months, although the usual interval was 1 to 4 months. It was found that the additional abnormalities seen in the chronic phase, most often a double Ph1 chromosome (5%) or +8 (2.4%) were also those most frequently seen in the blast phase. Also, the possible isochromosome for the long arm of 17 ([17q]) was considered to be a reliable marker for the blast phase, and other structural rearrangements independent of the Ph1 translocation occurred in about 10% of the patients.

156. Reports of patients with CML whose cells have been analysed with banding have included those who are Ph1-negative. The Ph1-negative patients account for about 18% of the cases studied in chronic phase [R12]. These patients tend to be older, with a large percentage of males. They show a smaller elevation of the white blood cell count than do Ph1-positives [W10], and respond poorly to treatment and chromosomal changes in their karyotype. In some instances, such changes preceded the onset of clinically apparent blast crisis by up to 18 months, although the usual interval was 1 to 4 months. It was found that the additional abnormalities seen in the chronic phase, most often a double Ph1 chromosome (5%) or +8 (2.4%) were also those most frequently seen in the blast phase. Also, the possible isochromosome for the long arm of 17 ([17q]) was considered to be a reliable marker for the blast phase, and other structural rearrangements independent of the Ph1 translocation occurred in about 10% of the patients.

157. In contrast to the observations discussed in the preceding section on CML in which a specific chromosomal abnormality, the Ph1 chromosome, is found in over 90% of the patients, the chromosome patterns in acute leukaemias (either myelogenous (AML) or lymphoblastic (ALL)) are quite variable, although chromosomal abnormalities have been detected in about 50% of the cases. Sandberg et al. [S36] first suggested that patients with AML are more likely to have diploid or hypodiploid chromosome numbers whereas those with ALL are more likely to have hypodiploid chromosome numbers. Although chromosomal changes in bone marrow cells of acute leukaemic patients are diverse, an extra C group chromosome (8 or 9 or sometimes 10 or 11) had been repeatedly reported in bone marrow cells of leukaemic patients as well as in patients with other haematopoietic disorders [J7, R15]; a missing or deleted chromosome 7 [P12, R16] or an isochromosome for the long arm of chromosome 17 has also been found [F7, M27].

158. Rowley [R12] recently analysed the banding patterns of chromosomes of 60 patients with AML, acute myelomonocytic leukaemia (AMMoL) or erythroleukaemia (EL) (see table 8 in reference [R12]). It was found that there is a surprisingly narrow range of modal chromosome numbers, with 22 individuals having 45 chromosomes, 16 having 46 and 15 having 47; 5 had 42–44 and 4 had 48–50 chromosomes; the chromosome abnormalities can be grouped into three major types: gain of one autosome, loss of one autosome and balanced translocation. It was further found that 13 patients had one extra autosome identified as number 8 in ten cases. Nine showed loss of one autosome, identified as number 7 in six cases. Five patients had an 8:21 translocation.

159. The summary report of van den Bergh et al. [D9] on acute non-lymphocytic leukaemia (ANLL) in 279 patients is in general confirmatory of Rowley's analysis. They found that out of the 279 patients with ANLL, 140 had an apparently normal karyotype and 139 were chromosomally abnormal. Among the latter group, 22 cases had +8, 20 cases were −7, 11 cases had t(9q−;21q+), 9 cases had t(15q−;17q−) and 5 cases were t(9q−;22q−). Of the remaining 139 patients 72 had modal chromosome numbers as follows: less than 46 (19 cases); equal to 46 (30 cases) and higher than 46 (23 cases). The survival of patients was related to the karyotypes of the marrow cells. Patients with only normal cells (NN), with a mixture of normal and abnormal cells (NA) and with only abnormal cells (AA) had median survival times of 6, 5 and 4 months, respectively. The correlation between karyotype and survival was most significant for patients with AML in which the median survival was 8 months for NN patients, but 2 and 3 months for AN and AA patients, respectively.

160. It would thus appear that AML and CML in the acute phases have some chromosomal changes in common, namely, an additional number 8 and, less frequently in the former, an i(17q). Other changes appear to be relatively specific for one disease or another. The Ph1 chromosome is restricted to CML and an extra F, shown to be number 19, is found in CML and not in AML. Loss of Y may occur in up to 10% of Ph1-positive males, but is rare in AML. The absence of number 7 or an X in females or the (8:21) translocation appears to be limited to AML [R12].

161. In 1967, Martineau [M28] reported the occurrence of a long submetacentric marker in 8 out of 9 patients with testicular tumours (seminomas) but so far no banding studies have been reported on these tumours.

162. Burkitt's lymphoma is a sporadic disease of children. First described in Africa, it is also known to occur in virtually epidemic proportions in New Guinea. It shows involvement of the peripheral lymph nodes (e.g., in the jaw) or with a lymphomatous mass in the lower abdomen. The course of the disease is usually short, resulting in death within a few years. Burkitt's lymphoma is now known to occur worldwide, including the Americas and Europe. In such areas, the children tend to be a little older when they contract the disease, to be more resistant to drug therapy and to die even more quickly after diagnosis than in Africa or in New Guinea [W6].

163. The most interesting difference between the African (and New Guinean) (AeBL) and the American types (AmBL) is related to the close association with the Epstein-Barr virus in the majority of the cases of the former type whereas this is not true of the latter. Biopsies and cell cultures from patients with AFB show an abnormal chromosome 14 with an additional terminal band [J8, M29] in many cases. Zech [Z4] and Zech et al. [Z12] have provided evidence suggesting that the extra R band at the distal end of the long arm of
chromosome 14 is the result of a balanced translocation
from chromosome 8, i.e., (8q-;14q+).

164. Studies of cells cultured from two children with
AmBL showed that in one there was no detectable
Epstein-Barr virus while in the other, it was present.
In both cases, the (8q-;14q+) was found and was indis-
tinguishable from that observed in the A/BL [E5].

(e) Meningiomas

165. Cytogenetic studies of human meningiomas have
shown that the majority have a hypodiploid cell line
and the G group chromosome missing in many cases is
number 22 [M30, Z5]. In addition, the loss of chromo-
somes 8, 9, X and Y have also been reported in a few
cases [M31, Z6].

(f) Ataxia telangiectasia

166. In blood lymphocytes and fibroblasts from
patients with AT, increased spontaneous chromosome
breakage has long been known [H21, H23, H29, G16,
L11]. More recently, chromosomal rearrangements of
the translocation type have been described in
association with chromosome breakage [B18, P14].
Bandings studies have shown the specific involvement
of chromosomes 7 and 14 in translocations, generating in
particular, t(14;14) and t(7;14) [H30, M32, O7, R17].
The involvement of chromosomes 7 or 14 with other
chromosomes has also been reported [M32, O7].

167. In their study, McCaw et al. [M32] found translo-
cations involving 14q in lymphocyte clones obtained
from 7 out of 8 AT patients; the other patient had a ring
14 chromosome. The breakpoints in chromosome 14
involved in the translocation were in the q12 band
whereas those in the recipient chromosomes were at
or near the end. The breakpoints and the extent of
probable deletion in the ring 14 chromosomes were not
determined. The authors had an opportunity to study
the chromosomes of one patient before and after the
onset of chronic lymphocytic leukaemia. Before
leukaemia was diagnosed, the patient had a lymphocyte
cloned with a 14q translocation in about 20% of the
lymphocytes sampled. After the onset of the leukaemia,
100% of the cells sampled from peripheral blood were
leukaemic and showed only one of the two number 14
chromosomes, namely one with extra material on its
long arm (14q+). The authors consider that the
evolution of the leukaemic clone from the pre-existing
translocation clone was not fortuitous and that the
leukaemic transformation was intimately related to the
structural rearrangement of 14q. They also believe that
the increasing evidence provided by others for the non-
random involvement of 14q in African-type Burkitt
lymphoma and other lymphoid neoplasms support their
hypothesis.

168. Auriás et al. [A9] have reported the results of a
study on R-banding of lymphocytes and fibroblasts
from 11 AT patients and 6 relatives (parents, siblings)
of the patients. Among a total of 927 lymphocytes
analysed, there were 158 chromosomal rearrangements
and out of 187 fibroblasts examined, 33 were chromo-
somally abnormal giving frequencies of 0.17% for
lymphocytes and 0.18% for fibroblasts. The most
frequent rearrangement is a pericentric inversion of
chromosome 7 and this is true of both lymphocytes and
fibroblasts. The relative frequencies of inv(7) and other
rearrangements are: 29,inv(7); 8,t(7q;14q); 9,t(7p;14q);
6,inv(14); 24 other rearrangements involving chromo-
somes 7 or 14; 104 rearrangements involving other
chromosomes and a few other rearrangements of a
complex type involving chromosomes 7 or 14. The 191
rearrangements detected corresponded to 316 recog-
nizable breakpoints, 35% of which involved chromo-
some 7, 15% involved chromosome 14, the remainder
involving other chromosomes. Among the 112 break-
points in chromosome 7, 41 seemed to affect band p14
and 40, band q35; an analogous non-random situation
existed with respect to chromosome 14: bands q12 and
q32.3 are predominantly affected.

169. These results thus demonstrate that inv(7) is the
most common single type of rearrangement in AT
patients and are at variance with those reported by
other investigators who had found t(14;14) and t(7;14)
to be the predominant types. The basis for the
difference is not clear at present, but may possibly be
related to methodological problems (the present work is
the first one to use R banding systematically for all cells
examined), geographical differences, age, severity of
the disease, etc.

170. Turning now to the results from the study of the
relatives of AT, in two of the three parents, 2 rearrange-
ments of chromosome 7 and 14 (one, an inv(7) and
another a t(7p;14q)) were found in 225 cells. Among 2
of the 3 siblings, 6 rearrangements of chromosomes 7
and 14 were found in 205 cells. In a further study of 17
AT heterozygotes, Auriás et al. [A9] confirmed the
above finding with respect to the specificity of
rearrangements and their rate of occurrence.

171. It is worth pointing out here that several investi-
gators have independently reported the occurrence of
t(7;14) in lymphocyte cultures of apparently normal
individuals [A10, B19, H31, W13, Z8]. The apparent
breakpoints on each of the two chromosomes were
nearly similar in all these cases (chromosome 14: q12 or
q1(2); chromosome 7: q13 or q15). Auriás et al. [A9]
stress that in most of the other studies on non-AT
patients, inversions had not been detected and this
could have been due to biased analyses. Inversions of
chromosome 7 and 14 were among the most common if
not the most common chromosomal change. Both in
cells from AT patients and in cells from presumed
normal individuals. In both categories the frequency of
inversions was probably underestimated because of the
difficulty in detecting them. Their data lend credence to
the possibility that in AT individuals and AT heterozy-
ogotes, the frequencies of rearrangements involving
chromosomes 7 and 14 may be, respectively, 40 and 9
times higher than in presumed normal cells.

172. The specific chromosomal defects in cancer
discussed in this section and some others not discussed
are summarized in Table 9.

3. Summary and conclusions

173. Many single gene traits (autosomal dominant,
autosomal recessive and X-linked) predispose to or are
complicated by neoplasia. The well studied ones are
exemplified by xeroderma pigmentosum (XP), ataxia
telangiectasia (AT), Fanconi's anaemia (FA), Bloom's
syndrome (BS) and retinoblastoma. All these except
retinoblastoma are inherited as autosomal recessives.
Conditions such as AT, FA and BS are collectively
referred to as "chromosome instability syndromes" on the basis of the fact that spontaneous breakage of the chromosomes occurs in peripheral blood lymphocytes and in fibroblasts from these patients cultured in vitro.

174. The most notable abnormality in XP is hypersensitivity of the skin to solar radiation and is reflected by pigmentation changes, elevated erythema and multiple neoplasms; basal and squamous cell carcinomas are the most prevalent types. Clinicians distinguish two forms of XP: the classical XP displaying skin (and ocular) complications only and neurological XP in which a wide range of central nervous system defects accompany the skin lesions. XP heterozygotes do not seem to be at any increased risk for developing cancers.

175. AT is a complex neurovascular and immunodeficiency syndrome with a predisposition to cancer of the lymphoreticular system. Increased spontaneous chromosome breakage is evident in lymphocytes and less strikingly, in fibroblasts. More recently chromosome rearrangements of the translocation type involving chromosomes 7 and 14 and inversions in chromosome 7 have been described. There is evidence that the presence of the translocation containing clones in peripheral blood lymphocytes may herald the development of cancer; in one AT patient with chronic lymphocytic leukaemia, the neoplastic lymphocytes appeared to descend directly from a pre-malignant clone marked by a 14q translocation. Heterozygotes for AT appear to be at an increased risk for the development of cancer.

176. The predominant clinical features of FA are haematological disturbances involving all elements of the bone marrow, diverse anatomical malformations, cutaneous lesions and growth retardation. The affected individuals usually die in childhood from excessive bleeding or overwhelming infection and those who survive to adulthood are prone to acute leukaemia (primarily myeloid), myelodysplasia and various cell carcinomas of mucocutaneous junctions surrounding the oral and anal cavities and hepatic adenoma. The modal karyotype is normal in FA (this also holds true for XP and AT). Both blood lymphocytes and dermal fibroblasts are characterized by a high spontaneous frequency of chromatid-type breaks and gaps. The chromatid rearrangements are mainly between non-homologous sites. Heterozygotes do not seem to be at an increased risk for cancer.

177. BS is characterized clinically by severe growth retardation, a telangiectatic erythema on exposed areas, increased sun-sensitivity and impairment of the immune system. The risk of cancer is increased in BS patients and about half of the cancers are leukaemias of the non-lymphocytic type. The cytogenetic hallmark of BS is the symmetrical quadriradial figure which is rarely seen in other chromosome instability syndromes. The cells from BS patients show highly elevated frequencies of sister chromatid exchanges in leucocytes, fibroblasts and in bone marrow cells. There are no data on whether heterozygotes are at an increased risk for cancer.

178. Retinoblastoma is a malignant eye tumour of children. About 60% of the retinoblastomas are unilateral and non-hereditary, 15%, unilateral and hereditary and 25%, bilateral and hereditary. The mode of inheritance of the hereditary variety of retinoblastoma is autosomal dominant with over 90% penetrance. Cytogenetic evidence suggests that the locus for retinoblastoma is on the long arm of chromosome 13 at band q14.

179. There is good evidence for the association between aniridia (an autosomal dominant trait), Wilms' tumour and urogenital abnormalities and a specific deletion of chromosome 11 (11p13) appears to cause aniridia with a 1 in 3 risk for the development of Wilms' tumour and an even greater risk for the development of mental retardation. The gene coding for the enzyme catalase has been mapped to the same region as that which is involved in the aniridia-Wilms' tumour association.

180. Over the last two decades, the thesis that some specific chromosomal changes may be involved in neoplasia has gained increasing support. Thus for instance, in chronic myeloid leukaemia, a translocation between chromosomes 9 and 22 has been diagnosed in a majority of the cases. In acute leukaemias, the chromosomal patterns are variable although chromosomal abnormalities (some specific) have been detected in about 50% of the cases. In Burkitt's lymphoma, the main chromosomal change seems to be a translocation involving chromosomes 8 and 14.

E. HUMAN DISORDERS SHOWING INCREASED SENSITIVITY TO THE INDUCTION OF GENETIC DAMAGE BY PHYSICAL AND CHEMICAL MUTAGENS: THE ROLE OF DNA REPAIR

181. In the 1977 report, some aspects of the sensitivity of cells derived from individuals suffering from certain inherited disorders to UV and ionizing radiation and the role of DNA repair processes were dealt with. This section will be devoted to an updating of the information in this area. For more extensive reviews, see [A11, A27, C22, C61, H32, P33, P36, P37, P38, S41, S42].

182. The disorders that have been extensively studied from the standpoint of increased sensitivity to DNA damaging agents are XP, AT, BS and FA. Besides, some information is available for some other disorders. The increased sensitivity of affected individuals to a physical or chemical agent has been a useful indicator of a possible cellular defect in the ability of the cells to recover from induced DNA damage. Thus for instance, the increased sun sensitivity and the finding that XP individuals developed skin cancer at early ages prompted Cleaver's work with XP cells. This led to the discovery that XP cells are deficient in DNA repair [C23] of UV-induced damage and catalysed the search for other disorders that may show repair defects, not only with respect to UV-induced damage but also with regard to damage induced by other mutagens.

1. Sensitivity at the individual level

183. It is now known that, in addition to XP, BS and Cockayne's syndrome patients show increased sun sensitivity although the repair defects may be of a different nature. The same is also true of FA patients. There are three reports [C24, G18, M33] of AT patients showing unusual radiosensitivity. Gotoff et al. [G18] reported a ten-year-old boy with AT and a malignant lymphoma who, after receiving a maximum dose of 3000 rad to the nasopharynx (out of a planned tumour dose of 4000 rad) was noted to show marked symptoms
of cutaneous erythema and clinical signs indicated deep tissue damage. Following his death eight months later autopsy revealed deep tissue necrosis and it was concluded that the radiation was directly responsible for his death. The second report [M33] concerned a nine-year-old boy with AT and Hodgkin's disease who received a partial dose of 2843 rad (out of a planned dose of 4000 rad) to the mediastinum. The patient developed severe oesophagitis, skin damage and respiratory problems and died four months later. Lastly, Cunliff et al. [C24] reported a seven-year-old boy with AT and a malignant lymphoma in the upper lobe of the right lung. After 20 Gy, dysphagia and erythema were noted and, at 30 Gy, the treatment was stopped because of worsening symptoms. He died three weeks later. Again, death appeared to be due to the radiation treatment.

184. There are reports (e.g., [J23]) of patients with basal cell naevus syndrome showing severe responses to radiation therapy (the syndrome is an autosomal dominant condition characterized by multiple basal naevi, which frequently develop into carcinomas and a variety of minor malformations). In the case of familial retinoblastoma, it is known that the gene carriers are susceptible to other tumours, especially osteogenic sarcoma; the latter may affect 1% of gene carriers [J10, K17]. When gene carriers are irradiated, this risk rises sharply; with very large doses of x-rays to the orbit, the incidence of osteogenic sarcoma of the orbit may rise to 30% [S51].

2. Sensitivity at the cellular level

185. A number of studies have been carried out to examine the sensitivity of cells derived from these patients to the lethal, chromosome-breaking and mutagenic effects of radiation and of chemicals. These studies have revealed that the patterns are complex and not all of them are amenable to simple interpretations as will be discussed below.

(a) Cell-killing effects

186. In the work of Arlett and Harcourt [A28], the gamma-ray sensitivity to killing of over 50 lines of human fibroblasts (obtained from individuals suffering from one or another of the diseases mentioned above) was examined. It was found that the normal sensitivity could be described by a range of D0 values of 0.97 to 1.80 Gy. All ten AT strains tested proved radiosensitive and gave a mean D0 value of 0.57 ± 0.15 Gy and these represent the most radiosensitive human skin fibroblasts currently available. Representative cell strains from familial retinoblastoma, FA, Hutchinson-Gilford progeria occupied positions of intermediate sensitivity, as did one of the two AT heterozygotes. Six XP cell strains together with two Cockayne's syndrome cell strains (all known to be sensitive to UV) fell in the normal range, indicating an absence of cross-sensitivity between UV and gamma-irradiation.

187. In the x-ray study of Weichselbaum et al. [W26], again involving over 50 cell strains, the sensitivity of six cell strains from normal individuals was described by D0 values in the range from 1.4 to 1.52 Gy with an overall range, based on the extremes of their standard errors, of 1.28 to 1.64 Gy. About three-quarters of those studied (including those derived from patients with one or another condition associated with a predisposition to malignancy) fell in this range. Cell strains identified as sensitive came from AT patients (D0: 0.46 to 0.52 Gy), progeria (D0: 0.96 to 1.39 Gy), the two genetic forms of retinoblastoma (D0: 0.94 to 0.98 Gy) and partial trisomy for chromosome 13 (D0: 0.75 to 0.95 Gy).

188. If the sensitivity of AT cell strains is expressed in terms of a dose-reduction factor (DRF) relative to normal strains, this corresponds to a value of about 3 [P36, P37, P38, P39]. This is true irrespective of whether the cells are irradiated underoxic or hypoxic conditions. AT cell strains also display a uniform response to inactivation by 14 MeV neutrons; however they are only from 1.6 to 2 times more sensitive to this densely ionizing radiation than are normal strains [P37, P38].

189. While AT strains are consistently hypersensitive to killing by ionizing radiation, their response to many chemical carcinogens is less uniform. AT strains in general seem to be sensitive to those chemicals whose biological effects mimic those of ionizing radiation such as methylmethane sulphonate (MMS) and N-methyl-N'-nitro-N-nitroguanidine (MNNG); AT cells are inactivated at a normal rate by far UV (chiefly 254 nm) light or UV-mimetic chemicals, such as N-acetoxy-acetamidofluorene (N-acetoxy-AAF) (see [A11, P37, P38] and references cited therein). There is however, much more interstrain variability in response to treatment with radiomimetic chemicals than is observed for ionizing radiation.

190. XP cells, with the exception of the XP variants, are very sensitive to the lethal effects of UV-irradiation (reviewed in [C25]). XP variants in contrast, are only slightly more sensitive than normal cells [L13]. XP strains display cross-sensitivity to certain chemical carcinogens, including reactive forms of polycyclic hydrocarbons (e.g., "K-region" epoxides of benzo(a)pyrene and aromatic amides (e.g., 4-NQO; [T12]) but respond normally to ionizing radiation, monofunctional alkylating agents (e.g., MNNG) and the DNA-cross-linking agent (mitomycin-C) [A18, F9, M38, S41].

191. The pattern of sensitivity of FA fibroblasts to different physical and chemical agents is somewhat different than that of XP or AT fibroblasts. FA cells are type II hypersensitive to the DNA intercalating agents (e.g., mitomycin-C, nitrogen mustard) or to psoralen-plus-black light, but are at most only slightly more sensitive to far-UV or gamma-irradiation, 4-NQO or MMS [F9, S104].

192. Fibroblasts from BS patients are not unusually sensitive to UV- or x-ray-induced killing, but there are results [K13] showing that BS lymphocytes may be highly sensitive to EMS-induced killing. Fibroblasts from Cockayne's syndrome patients are also hypersensitive to UV-induced killing [M37, S45] but show normal sensitivity with respect to x-rays.

193. Some of the main findings discussed in this subsection are summarized in Table 10.

(b) Host-cell reactivation

194. Several cell strains derived from patients with hereditary disorders such as XP, AT, etc., have been assayed for ability to support the reproduction of mutant-inactivated viruses, a phenomenon known as
host-cell reactivation (HCR). The XP cells have a reduced capacity to reactivate UV-irradiated adenovirus 2 assayed on the basis of either plaque-forming activity [D21] or production of viral structural antigens [R67]. Similar results have been obtained with UV-irradiated SV-40 virus, herpes simplex and vaccinia virus [A15, A16, L15; see also S105]. Several AT strains reactivate MNNG or x-ray-damaged adenovirus normally [D22, R67] despite their hypersensitivity to killing by these two agents; however, HCR of far-UV-irradiated virus is slightly reduced, using both plaque-forming ability and synthesis of viral (V antigen) protein as end-points [R67] although AT cells are inactivated at normal rates by far-UV light. These observations suggest that repair functions presumed to be deficient in AT cells are not required to promote survival of x-ray- or MNNG-damaged virus but are required to assist in viral recovery from a component of far-UV damage to DNA. There is some evidence for progeroid fibroblasts showing a reduced capacity to reactivate gamma-irradiated adenovirus [R69] and for FA cells showing a reduced capacity to produce viral structural antigens after infection with UV or gamma-irradiated adenovirus [R68].

(c) Mutation induction

195. Maher et al. [M35, M38, M39] have shown that UV irradiation induces significantly higher frequencies of 8-AG resistant mutations in both classical XP and variant XP cells than in normal cells. Likewise, the XP cells were found to be more susceptible (by a factor of 2 to 3) to the induction of mutations by the “K-region” epoxide of benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene and dibenz(a,h)anthracene; the XP variant cells (from a patient) were also found to be more sensitive to mutation induction by hydrocarbon epoxides [M34]. Thus, at least in the case of the XP cells, there is a reasonably good correlation between cell killing and mutagenic effects of UV. The preliminary results of different investigators mentioned by Arlett and Lehman [A11] for AT cells suggest, in contrast, that these cells may be hypomutable or even immutable by ionizing radiation. A similar lack of correlation between sensitivity to killing and to mutation induction is also observed in the case of AT cells treated with mitomycin-C. It is tempting to speculate that error-prone repair pathways which in normal cells give rise to gamma-ray or mitomycin-C induced mutations may be inoperative in the AT and FA cells, respectively.

(d) Induction of chromosome aberrations and sister-chromatid exchanges

196. XP strains display more chromosome breakage than normal cells upon treatment with UV light or “UV-like” carcinogens but not with “ionizing-radiation-like” chemicals [B13, S43, S106, W17]. The chromosomal sensitivity to UV appears to be insufficient to account for the increased lethality, suggesting that the chromosome aberrations are probably not the principal cause of cell death in XP [M36]. Most, but not all, XP cells show an elevated rate of UV-induced SCEs [D12, P15, S47]. Wolff and co-workers [W17] reported that a virus-transformed XP cell strain from complementation group A showed increased induction of SCEs by a variety of chemical mutagens, even those (such as EMS) to which XP cells exhibit a normal repair and cell-killing responses. The results of Perry et al. [P15] support those of Wolff and co-workers. Heddle [H34] found that elevated levels of SCE induction by EMS were not obtained in three untransformed XP cell strains from complementation group A. In XP variants, UV exposure results in normal levels of SCE [D12, W17].

197. Ionizing radiation produces a much higher frequency of chromosomal aberrations in AT than in normal cells; furthermore, it has been shown that lymphocytes of AT patients when irradiated in G0 or G1 show both chromosome-type and chromatid-type aberrations, in contrast to the normal situation of only chromosome-type aberrations [T7]. Natarajan and Meijers [N12] studied the x-ray-induction of chromosome aberrations in peripheral blood lymphocytes as well as in skin fibroblasts from AT patients and found that in G0, G1 and G2, more aberrations were induced in AT cells than in normal cells. These results as well as those of Taylor [T9] demonstrate that base damage which needs an S-dependent repair is not entirely responsible for the increased frequency of x-ray-induced aberrations in AT cells. The frequencies of SCEs induced by x rays (as well as those induced by a number of chemical mutagens) are in the normal range [G20].

198. FA cells show an extreme specific sensitivity to both the lethal and chromosome-breaking effects of DNA cross-linking agents such as mitomycin-C [F8, F9, S44]. The induction of chromatid-type aberrations by mitomycin-C is markedly elevated in FA lymphocytes [S44] and this is also true of x-ray exposure [H56]. Diepoxybutane, another bifunctional agent, also increases the yield of chromosome aberrations at concentrations that produce no increase in normal cells [A14]. In FA cells, a lower than normal number of SCEs are induced in blood cells (but not fibroblasts) by mitomycin-C [L16].

199. It has been reported that cells (erythrocytes) from two unrelated FA patients are deficient in superoxide dismutase [J9] and that treatment with this enzyme reduces the spontaneous level of chromosome breakage in cultured cells [N13]. Raj and Heddle [R19] tested whether or not treatment with superoxide dismutase (among other enzymes chosen) will lead to a greater proportional reduction in chromosomal damage (measured using the induction of micronuclei as the criterion) of mitomycin-C treated FA fibroblasts. The results showed that while the enzyme treatment reduced both the spontaneous and mitomycin-C-induced chromosome breakage, there was no consistent pronounced effect in FA cells.

200. BS cells do not show increased frequencies of chromosome aberrations following UV- or gamma-irradiation [15] but they seem to show an elevated SCE response following exposure to EMS [K13]. Cockayne’s syndrome cells show an increased sensitivity to the UV-induction of SCEs [M36, S45]. In the study of Marshall et al. [M36], at any UV dose, approximately 2.5 times more SCEs were induced in Cockayne’s syndrome cells than in normal fibroblasts.

201. Some of the main results pertaining to chromosome aberrations and SCEs are summarized in Table 11.
3. DNA repair

(a) _Xeroderma pigmentosum (XP)_

202. There are now extensive biochemical and biophysical data on the DNA repair properties of UV-irradiated XP strains to support the idea that the abnormal UV response of XP cells is due to a molecular defect in the repair of UV-induced DNA damage. The defect is not complete, however, but may be over 90\% in the cells of some individuals and only 50\% in others [C25, K8, S41]. Cell strains established from all neurological XP patients and from most classical XP patients are deficient, to varying extents, in excision repair. This conclusion is supported by data on several molecular end-points: removal of dimers [C25, C26], disappearance of UV-endonuclease sensitive sites [F39] and repair synthesis levels as monitored directly by unscheduled DNA synthesis (UDS) or repair replication [C26] or indirectly by photolysis of incorporated bromodeoxyuridine (BrdUrd) [R70].

203. The XP strains that are deficient in excision repair have been subdivided on the basis of complementation analysis, using the technique of somatic cell hybridization [K14]. Two strains are assigned to different complementation groups if, upon fusion, both nuclei of binuclear hybrid cells exhibit near-normal levels of UV-induced UDS. Alternatively, if the UDS levels remain reduced in the hybrids, the two strains are allocated to the same complementation group. Thus far, seven complementation groups have been identified [A17, D13, D14, K14, K15]. The strains designated as XP variants constitute a minority of the strains from persons having the classical form of XP; such strains are proficient in excision repair, but are deficient in post-replication repair after UV treatment [L12]. These variants like the excision repair strains possess reduced levels of photoactivity [S107] although the residual levels vary between different variant strains. Table 12 summarizes some of the main DNA repair properties of different genetic forms of XP.

204. There is considerable evidence for the thesis that the defect in most, if not all, of these groups is in the incision step. Firstly, following UV-irradiation of XP cells of groups A–D, only small numbers of single strand breaks (SSB) are observed compared with the number of dimers in the DNA [C26, F10]. Secondly, when T4 endonuclease V is introduced into UV-irradiated XP cells of groups A–E, UDS approaches normal levels and the introduced enzyme increases survival [T10, T11]. Thirdly, Ciarrocchi and Linn [C27] showed that T4 endonuclease restores repair replication activity in a cell-free system obtained from XP–A cells. Smith and Hanawalt [S47] characterized repair replication activity in isolated human cell nuclei, and demonstrated that the activity restored to XP–A cells by addition of T4 endonuclease closely resembled repair replication in normal human cells. The observation that the incision endonuclease can allow cells from several different complementation groups to perform the subsequent steps in excision repair suggests that all are deficient in incision but not in excision-resynthesis.

205. It would thus appear that mutation in any of a number of genes might lead to the loss of incision activity in human cells. However, the existence of seven complementation groups in XP does not necessarily indicate that seven different proteins are required for incision. Some of the groups may represent genes determining polypeptides that interact to form a single functional complex; others may reflect intragenic complementation, mutations in regulatory genes or mutations in genes whose products facilitate the endonucleolytic event [H32].

206. Some support for the last possibility mentioned above comes from the work of Mortelmans et al. [N40]. They found that normal human cells disrupted by sonication were capable of specifically excising pyrimidine dimers both from purified DNA that had been heavily irradiated, and from their endogenous cellular DNA. However, sonicated XP cells from groups A, C and D also excised dimers from purified DNA; but sonicated XP–A cells were unable to excise dimers from their endogenous DNA. This was shown to be a deficiency in enzyme activity rather than a property of the endogenous DNA, as DNA-free sonicates of normal cells were able to promote dimer excision from the XP–A DNA. These findings led to the suggestion that these XP cells are not deficient in the endonuclease activity per se but in some other activity necessary for incision in vivo. Alternatively, the excision of dimers from purified DNA may represent the activity of enzymes not normally associated with excision repair in vivo. Although the inability of sonicated XP–A cells to excise dimers from their endogenous DNA mimics their excision properties in vivo, this is not true of XP–D cells in which excision is observed in vitro but not in vivo.

207. It was mentioned earlier that cells of the XP variant class have normal or near-normal sensitivity to UV light and normal excision repair. These have a defect in post-replication repair [L12], a process which may be defined as “the ability of UV-irradiated cells to achieve the eventual synthesis of high molecular weight daughter strands of DNA despite the presence of unexcised damage in the template strands” [A11]. It has been demonstrated that the molecular weight of newly synthesized DNA in UV-irradiated XP variants is considerably lower than in normal cells [L12]. This suggests that gaps in the daughter DNA strands (presumed to be opposite damage in the template strands) persist for much longer in XP variants than in normal cells. In addition, caffeine has been found to inhibit the sealing of these gaps in XP variants, but to be without effect on normal cells [L12].

208. Park and Cleaver [P16] studied DNA synthesis in normal cells and in excision-defective (group A) and XP-variant cells after irradiation with UV. The sizes of DNA synthesized during brief pulses of tritiated thymidine 1–2 h after irradiation were decreased, the XP variant showing the smallest molecular weight. Once synthesized, however, the labelled DNA increased in size at the same rate as in control in all strains, and the rate was relatively insensitive to caffeine. After 2–3 h, labelled DNA in each cell type reached a maximum size that was less than in the control cells, indicating the presence of long-lived blocks to DNA chain growth. These authors argue that in the studies of Lehman et al. [L12, L13] where one or two chase times were used, the major difference between XP variant cells and normal cells was expressed in the size of the labelled DNA made, not on its subsequent rate of elongation. On the basis of their results, the authors proposed a model alternative to the post-replication repair model; their model assumes normal chain elongation and termination mechanisms in which the dimers and other damaged sites act as all-or-nothing blocks to the progress of the replication forks. Therefore, although the XP variant has a unique
response to UV damage, this does not involve a defect in the bypassing of dimers during a post-labelling chase (i.e., no gaps opposite dimers) but only blocked forks at dimer sites (see also [C61]).

209. The mutation frequency induced by UV in XP variants is well above normal not only when expressed per unit UV fluence but also per survivor [M38, M39]. In contrast, the XP strains deficient in excision repair are only hypermutable when the mutation frequency is given as a function of UV fluence and not survival [M38]. These observations provide good biological evidence that post-replication repair is relatively error-prone whereas, excision repair is error-free, at least in response to UV damage.

210. The fact that all XP cell lines are defective in one or more repair pathways for UV damage have led to the speculation that UV-induced skin carcinogenesis in normal human beings has a low probability because most of the lesions are removed and DNA synthesis beyond any that remain is relatively error-free. In excision-defective XP cells, many dimers remain and replication beyond them, although relatively error-free, makes an appreciable number of mistakes and hence gives a high possibility for neoplastic transformation. In the XP variants, there are a few more dimers than in normal cells and the level of damage left after DNA repair synthesis is also due to the absence of normal repair, but replication beyond dimers is error-prone and, as in the case of conventional XP's, an appreciable number of mistakes is made [S41].

211. XP cells are also defective in the repair of damage induced by a number of chemical agents while they are repair-proficient with respect to the damage induced by certain other chemicals, as well as by ionizing radiation: these are listed in Table 13. It should be realized that the classification of chemical compounds as making repairable or irreparable damage in XP cells, although useful, is complicated by the fact that most, if not all, agents each produce a number of different DNA lesions. The repair of a fraction of these lesions can be impaired in XP cells while the remainder is normal. This seems to be the case with alklylation damage to DNA. Both normal and XP cells are able to remove readily N7-alkylguanine, but only normal cells can do it for the minor, but biologically more important product O6-alkylguanine [G21].

212. Earlier studies with 4-NQO demonstrated that XP cells were more sensitive than normal cells for cytotoxic effects [T12] and that XP cells had a relatively low level of DNA repair synthesis [C28, S48]. Ikenaga et al. [I2] found that three major stable purine adducts formed by 4-NQO treatment were removed from the DNA of normal cells but such removal was not detectable with the SV-40 transformed XP strain of complementation group A; a less stable guanine adduct however, did disappear from XP DNA, but the rate of removal was lower than in normal cells.

213. The similarity of effects of UV and 4-NQO in XP prompted Zelle [Z9] and Zelle and Bootsma [Z10] to investigate the problem in greater detail. The hypothesis was that if 4-NQO lesions are (predominantly) removed by the same pathway as the pyrimidine dimers and are dependent on the same gene products, then the classification of the different XP strains into different complementation groups should be similar with respect to UV and 4-NQO repair in the strains. DNA repair was studied by determining the extent of UDS in the exposed cells by means of autoradiography. The strains that are classified into the same complementation group on the basis of their repair of UV-damage also did not complement each other after 4-NQO treatment. Strains belonging to different complementation groups (because they do complement each other for the excision of UV lesions), also showed complementation for the repair of 4-NQO-induced damage. In the pure strains, the degree to which UDS after 4-NQO treatment was depressed followed the same pattern as was seen for the repair of UV-damage. From these results, the authors have drawn the conclusion that repair pathways of UV and 4-NQO lesions have steps in common and that the gene products responsible for the repair deficiency in the groups A, B, C, and D are of equal importance for the repair of the majority of the damage induced by 4-NQO.

(b) Ataxia telangiectasia

214. The DNA repair characteristics of AT fibroblast strains after irradiation or other mutagen treatments are summarized in Table 14. The 13 strains examined thus far, can be divided into two broad categories. denoted as exr- and exr+, on the basis of their capacity (relative to that of normal strains) to execute DNA repair synthesis (i.e., DNA repair replication and UDS) following gamma-irradiation or [P37]. The diminished level of gamma-ray-induced repair synthesis observed in the exr- strains does not seem to stem from a defect in strand-rejoining, because all AT strains reconstitute both single-strand (including alkali-labile lesions) and double-strand-breaks with normal kinetics, as judged by several independent criteria [L17, P18, T7]. Instead, the reduction in repair synthesis can be ascribed to a defective capacity to remove alkali-stable radio-products which are detected as sites in DNA sensitive to the strand-incising activity of lesion recognizing enzymes present in crude extracts from Micrococcus luteus [P18, P36].

215. The site removal data imply that certain AT strains lack a fully functional enzyme (endonuclease or DNA glycosylase) or co-factor involved in the initial incision reaction in an excision-repair process. The radioproducts whose removal is presumed to be defective in exr- AT strains have not been identified: the whole spectrum of base, sugar and cross-link lesions are all candidates with the exception of thymine glycols. AT strains, both exr+ and exr-, apparently repair this numerically important class of modified bases normally [C62, R71].

216. The pattern of DNA repair replication for six AT strains when treated with MNNG resembles that observed after exposure to hypoxic gamma-irradiation: four exr- strains exhibit diminished levels and two exr+ strains, normal levels [S110]. Recently, Lehman et al. [L45] reported that they were unable to confirm the above observations of Scudiere [S110] regarding defective repair synthesis in AT3B1 (exr-).

217. In spite of the enhanced sensitivity of some AT strains to the lethal effects of MMS, all strains have an apparently normal capacity to repair damage caused by this alkylating agent, at least as reflected by DNA repair replication. In agreement with cell survival studies, the AT cells are proficient in the repair of far-UV light [A18, P17] and AAF-induced [A19] damage.

218. Although considerable success has been achieved in elucidating the DNA properties in AT strains, the
precise biochemical defect responsible for the enhanced radiosensitivity observed at the cellular and cytogenetic levels remains unknown. Enzymological studies have provided little insight into the nature of the underlying defect: cell extracts of both exr+ and exr- strains contain normal levels of activity of uracil-DNA glycosylase [K36] and AP endonuclease [IS3, M62, S111].

219. Inoue et al. [13, 17] have shown that the ability of cell-free extracts to increase the priming activity (in a DNA-polymerase assay) of gamma-irradiated DNA is severely reduced in several AT strains. A comparison in a similar assay of three AT homozygotes, one heterozygote and normal strains showed that homozygotes had substantially lower activity than normal strains, but no difference between heterozygotes and normal strains was found.

220. Cell fusion analyses have allocated three exr-strains to two complementation groups: AT1BE and AT3B1 to group A and AT 2BE to group B [P17; see also 17]. AT is thus genetically heterogeneous as is XP.

(c) Bloom's syndrome

221. The nature of the DNA repair defect in BS is not known, but Hand and German [H35] have demonstrated a lowered rate of DNA chain growth in S-phase fibroblasts which may account for the observation that the sedimentation of pulse-labelled DNA after UV-irradiation is somewhat slower than in normal cells [G22].

(d) Fanconi's anaemia

222. The unusually high sensitivity of FA cells to DNA cross-linking agents has already been mentioned. It has been suggested that FA cells are defective in the repair of DNA cross-links [F9, S20]. The DNA of FA cells analysed on alkaline gradients immediately after treatment with mitomycin-C sediments more rapidly than that from normal cells [F9]. This fact may be consistent with the explanation offered by Latt et al. [L16], namely, that mitomycin-C damages one polynucleotide chain (as do monofunctional alkylating agents) but removal of the fragment connected to the other chain by an inter-strand cross-link cannot be effected normally. The steps for the repair of a fragment in mammalian cells are not defined and this is true also of the fundamental molecular lesion in FA. Poon et al. [P18] observed defective removal of thymine dimers following large UV doses; with 4-NQO, normal endonucleolytic strand scission, and normal levels of UDS were obtained. Remsen and Cerutti [R72] found that in two out of four FA strains, the ability of cell nuclear preparations to excise thymine glycols from gamma-irradiated exogenous DNA was reduced. For all FA strains thus far investigated, the repair of x-ray-induced single-strand breaks is not deficient. A recent report [K16] has described a significant decrease in DNA ligase activity observed in fibroblasts and lymphocytes of a patient with FA and his mother (following UV-irradiation). No differences were found in the other steps of repair.

(e) Cockayne's syndrome

223. The nature of the molecular defect in Cockayne's syndrome is not yet known. Wade et al. [W18] have reported that the fibroblasts derived from seven Cockayne's syndrome patients show increased UV- but not x-ray sensitivity; reduced amounts of UDS following UV-irradiation; reduced incorporation of H-thymidine into small molecular weight single-stranded DNA after UV; normal excision of UV-induced pyrimidine dimers; and complementation of the ability to repair UV-induced DNA damage in cell hybrids formed between some of the fibroblast strains. Assays on crude extracts of 5 of the 7 strains examined thus far show that all 5 contain less than 50% of the normal DNA polymerase activity.

4. Summary and conclusions

224. The best studied human genetic disorders from the standpoint of sensitivity to mutagens (both at the individual and at the cellular levels) and DNA repair aspects are xeroderma pigmentosum (XP), ataxia telangectasia (AT), Fanconi's anaemia (FA), Bloom's syndrome (BS) and Cockayne's syndrome. XP, BS and Cockayne's syndrome patients show increased sensitivity to and all patients are highly sensitive to ionizing radiation.

225. XP cells (with the exception of the so-called variants) are very sensitive to the killing effects of UV and of certain chemicals such as some derivatives of acetylaminooulofluorene (AAF) and nitroquinoline oxide (4-NQO) which form large adducts to DNA. Their response to ionizing radiation and to relatively simple alkylating agents such as MMS and EMS is normal. XP cells are deficient in host-cell-reactivation of viruses. XP cells are also more sensitive to mutation induction (8-AGA) by UV and chemicals such as the "K-region" epoxide of benzo(a)pyrene, 7, 12-dimethyl-benz(a)-anthracene and to the UV-induction of chromosome-breakage events and sister-chromatid exchanges (SCEs).

226. There is now a substantial body of evidence supporting the premise that the XP strains are defective in the repair of UV-induced DNA damage: all strains (with the exception of the XP variants) are deficient, to varying extents, in excision repair. The variant strain is excision-repair proficient, but post-replication repair deficient.

227. When unscheduled DNA synthesis (UDS) after UV irradiation was examined in the nuclei of heterokaryons formed by fusing cells from different XP patients (their cells being deficient in excision repair), certain combinations appeared normal in excision repair capacity. On this basis, seven complementation groups designated A to G have been defined.

228. The AT cells represent the most radiosensitive (to ionizing radiation) human cells known but their response to many chemical carcinogens is not uniform. In general, the AT cells are more sensitive to those chemicals whose biological effects mimic those of ionizing radiation. Host-cell reactivation as well as response to far-UV are normal in AT cells. Preliminary results suggest that AT cells are less mutable than normal cells by ionizing radiation.

229. Ionizing radiation produces a much higher frequency of chromosomal aberrations in AT than in normal cells. AT cells display an unusual pattern of chromosome aberrations following irradiation in either G0 or early G1 phases in the sense that the aberrations
produced are both chromosome-type and chromatid-type. On the basis of their capacity to perform UDS following hypoxic gamma-irradiation (relative to normal cells), the AT strains can be divided into two broad categories, those which are excision-repair proficient and those which are deficient in this respect. Three excision-repair deficient strains have been allocated to two complementation groups A and B, on the basis of complementation studies. The precise biochemical defect responsible for the enhanced radiosensitivity observed at the cellular and cytogenetic levels still remains unknown.

230. FA cells are hypersensitive to bifunctional alkylating agents and to psoralen-plus-black light, but are only slightly more sensitive than normal cells to far-UV, gamma-irradiation, 4-NQO or MMS. The induction of chromatid aberrations by mitomycin-C is elevated in FA lymphocytes and this is also true of x-ray exposure; diepoxybutane also elicits a higher response in FA cells.

231. Fibroblasts from BS patients are not unusually sensitive to UV or x-ray-induced killing, but there are some data showing that BS lymphocytes may be more sensitive to EMS-induced killing. Fibroblasts from Cockayne's syndrome patients are hypersensitive to UV-induced killing, but show normal sensitivity with respect to x-rays. There are some data which suggest that some Cockayne's syndrome strains may have reduced levels of DNA polymerase activity (in crude extracts).

F. OTHER RELEVANT DATA

232. Although a detailed review of the data bearing on the radiation-induction of chromosome aberrations in somatic cells per se is not within the scope of this Annex, the following data are discussed in view of their topical interest and importance.

1. Chromosome aberrations in lymphocytes of individuals living in an area of high radioactivity

233. Pohl-Rüling and Fischer [P20] have summarized the results of their studies on chromosome aberrations in peripheral blood lymphocytes of individuals living and working in Badgastein, Austria, an area known to have a high natural radioactivity. The thermal radon-containing springs constitute the main source of this radioactivity. Five million litres of hot water containing high levels of radon are delivered daily and most of the water is conducted to big reservoirs and from there to hotels and spa houses where it is used for treatment. Almost all of the 222Rn is discharged into the air. In addition, radon emanates from the ground in the whole region. The air activity is lower in the periphery (zone I: 80–150 mR h−1, open air; 100–190 mR h−1, room air) than in the vicinity of the springs (zone I: 80–170 mR h−1, open air; 120–300 mR h−1, room air). Higher levels occur in the various rooms with treatment facilities and the highest air activity is found in the "thermal gallery" 1500 mR h−1, a former gold mine near Badgastein in which more than 5000 patients are treated per year. The radiation burden of the population is derived from inhaled radon and daughters in addition to external gamma irradiation. However, the alpha dose differs widely, being dependent on site of habitation and occupation and type of work. The total dose is lower for inhabitants of zone II than for inhabitants of zone I.

234. Chromosome studies were carried out in 180 blood samples from 122 persons grouped into five categories with increasing radiation doses, according to their geographical position within the Badgastein area and their occupations in the thermal baths, spa house or thermal gallery. Category A comprised members of the population living and working in Badgastein and its surroundings who were continuously irradiated by the background radiation; category B consisted of individuals who received occupational irradiation daily six times per week for 4 to 6 h (B1, bath attendants) or 8 to 10 h (B2, thermal gallery personnel) in addition to the Gastien area background irradiation; category C included doctors and train drivers (miners) who received the dose pattern B2 and in addition a high alpha dose six times per week during one (C1) or two (C2) 2 h period of duty within the mine. In addition, there were other individuals who were considered a special group (caretaker who lived on the premises and others who had received diagnostic irradiation etc.). The different groups of individuals studied had accumulated "blood burdens" of 110 to 340 mR h−1 of gamma ray dose and 1 to 1600 mR h−1 of alpha dose.

235. The main findings are:

(a) Even at these very low dose levels, dose-effect relationships were observed with the mean values of aberration frequencies (fragments, dicentrics and interstitial deletions) increasing from A to C;

(b) There was a weak dependence of chromosome aberrations on both age and dose only within groups A and B;

(c) The frequency of fragments showed an age-dependence for all groups. When these were normalized to an age of 50 years, the age-corrected mean values increased from groups A to C, although the dose-response patterns within each group showed differences (the slope was flatter in C than in A and B). At comparable doses, the number of fragments was higher in group A than in B and in B than in C. Within A, there was a strong dependence on alpha dose (expressed as mR per month), but no gamma-ray dose dependence. In group B, a weak dependence on both dose components was present and in group C, only to the gamma-ray dose;

(d) The results for dicentrics and interstitial deletions within groups A and B were similar to those for the fragments, namely, alpha dose dependence in group A and dependence on both alpha- and gamma-ray dose in group B. No linear relationship between dose and two-break aberrations could be established for group C as a whole although a weak alpha dose dependence was present in group C1 (in group C2, there was a decrease in aberration frequencies at the highest dose levels).

2. Chromosome aberrations in lymphocytes of nuclear dockyard workers

236. Evans et al. [E6] published the results of a study on the incidence of chromosome aberrations in peripheral blood lymphocytes of 197 dockyard workers followed up over a 10-year period. These workers had been exposed to mixed neutron + gamma irradiation during the refuelling of nuclear reactors, but most
exposures were below the internationally accepted maximum permissible level of 0.05 Sv per year. Details about the study population and methods of analysis are summarized in the following paragraphs. When the facility for refueling of nuclear-powered submarines was started in England in 1968:

(a) The majority of the workers who joined the establishment had not received previous occupational exposure to radiation;

(b) Blood samples were taken before their classification as "radiation workers" to give background control samples and serial blood samples were later obtained at periodic intervals as the dose accumulated;

(c) The blood was cultured using procedures standard to their laboratory; the cells were orcinol-stained and those with 45 or more centromeres were scored for dicentrics, rings, acentric fragments, minutes, abnormal mononuclei, additional or absent mononuclei (aneuploidy), a medium-sized chromosome with abnormal centromere separation (almost certainly an abnormal X chromosome) and chromatid aberrations;

(d) Dose estimates in rems, from film badges, were provided by the Admiralty Radiation Records Centre;

(e) The radiation sources were nuclear submarines undergoing refit and emitting mixed neutron + gamma radiation, although the exposures were stated to involve "almost exclusively gamma radiation".

237. The main results are:

(a) When the cumulative doses over the ten-year period were grouped into 0.05 Sv intervals, there was a positive correlation between the incidence of cells containing all types of aberrations with dose;

(b) In view of evidence from a variety of populations for an increase in the spontaneous frequency of aberrations (in the absence of known radiation exposure) with increasing age it was expected (and in fact found) that older workers and samples from later years contributed more to the higher dose categories, i.e., there was a positive correlation between dose and age, and dose and year of culture and aberration frequency;

(c) In view of the evidence from radiotherapy patients and from individuals accidentally exposed to high doses of radiation that the frequency of unstable aberrations decreases with time following the exposures, it was expected that, for a given time of blood sampling, an exposure a few days or weeks prior to sampling would result in higher detected incidence of induced unstable aberrations than an equivalent exposure received a year or more prior to sampling; this was in fact found;

(d) When all these considerations were taken into account, significant effects of dose were evident for the incidence of dicentric aberrations, acentric fragments and cells with unstable aberrations (Cu cells), but not for cells with symmetrical rearrangements: for the former type of aberrations (Cu), all data are consistent with a linear dose response;

(e) A significant age-effect (but no dose-effect) was found for aneuploid cells, for chromatid aberrations and for X chromosomes with abnormal centromere separation, as expected (the majority of peripheral lymphocytes are G1 at the time of exposure and consequently no chromatid type of aberrations would be expected);

(f) For all categories of Cu aberrations, the dependence on "recent dose" is greater, although not significantly so, than on "early dose". These data are compatible with the conclusion that considered overall, the rate of increase of dicentric aberrations is 1.4 $10^{-4}$ dicentrics per cell per 0.01 Sv.

238. The authors have stressed the point that the population examined is small in number, subject to very low levels of radiation exposure for periods of up to a maximum of ten years and is therefore unlikely to provide useful data on the incidence of malignant disease. But it is clear that the yield of dicentric aberrations in cells from individuals obtained prior to occupational exposure (or following exposure to less than 0.01 Sv) was about 1 in 700 cells rising approximately four-fold after accumulated doses of 0.2 to 0.3 Sv; the observed increase is not large, but is believed to be a direct expression of damage to genetic material consequent to radiation exposure. As the relationship between the yield of dicentrics in blood lymphocytes and reciprocal translocations in the germ cells varies between species, these data cannot also be used for predicting genetic hazards in the progeny of exposed individuals.

3. Chromosome aberrations in lymphocytes of nuclear power plant workers

239. In a study similar to that of Evans et al., Bauchinger et al. [65] made chromosome analyses of 57 healthy male employees of six German nuclear power plants; they were metal workers, technical engineers or radiation protection workers mainly in maintenance or refuelling crews. All of them had received annual doses below the maximum permissible limit of 0.05 Sv a--1 and had worked with radiation for periods ranging from 1 to 14 years. The exposure was mainly to external sources of gamma rays and higher energy x rays. The controls for this study were 11 healthy males with no radiation history except natural background.

240. The frequencies of dicentrics and acentrics in the radiation workers were significantly higher than in the controls; and there was no evidence for a positive correlation between the aberration yields and the accumulated total dose, even when only the recent annual dose of the workers was considered. Furthermore, multiple regression analysis of aberration frequencies on dose and age did not show any significant dependence on these. These observations are thus not in agreement with the findings of Evans et al. In addition, although the total dose-ranges were similar in these studies, the observed frequencies of aberrations (the data arranged in 0.05 Sv intervals) in the nuclear power plant workers were in general lower than those in the dockyard workers. The authors attribute the different nature of their findings to a combination of several factors such as the limited number of individuals studied, lack of adequate data on the dose accumulation patterns in the years preceding blood sampling etc.

4. Chromosome aberrations in lymphocytes of classified radiation workers

241. Lloyd et al. [49] have also compared the frequencies of dicentric and acentric aberrations in unirradiated control subjects and classified radiation workers exposed to gamma irradiation within permis-
sible limits at a nuclear establishment in the United
Kingdom and routinely monitored with film badges
and thermoluminescent dosimeters. All those exposed
had consistently recorded doses in the range of 0.015 to
0.05 Sv per year for at least the four years immediately
preceding sampling, and in many cases for more than
ten years. The incidence of both dicentrics and
acentrics was significantly higher in the exposed
workers than in the controls. When allowance was
made for the turnover of lymphocytes for the period
over which each worker had worked with radiation, a
linear dose-response relationship was found. The rate
of induction of dicentrics was (2.22 ± 0.94) 10^{-3} per
10^{-2} Gy and for all unstable aberrations, (8.24 ± 2.8)
10^{-4} per 10^{-2} Gy. These rates are in reasonable
agreement with dose-response data obtained in vitro.

5. Chromosome aberrations in lymphocytes of uranium
miners

242. Brandom et al. [B66] have studied the incidence of
chromosome aberrations in peripheral blood
lymphocytes of 83 underground uranium miners and 70
controls (in Colorado). The exposure estimates were
based on mine-air measurements and underground
working time. One working level (WL) is defined as any
combination of radon and radon daughters in 1 litre of
mine air which will result in the emission of 1.3 10^{13}
MeV of alpha-particle energy. One working level
month (WLM) is 1 WL times 170 working hours. Alpha
particles from short-lived 212Po and 214Po deliver the
main radiation dose to the tracheobronchial epithelium
and excess long-lived 210Pb in bone and blood is
reported to be related to the WLM estimates.

243. The difference between miners and controls in
the incidence of dicentrics and rings is not significant
although, collectively, there is a three-fold increase in
the frequency of these aberrations in the lymphocytes
of the miners. The frequencies of inversions and trans-
locations, as well as those of terminal and interstitial
deletions were higher in the miners. The frequencies of
all aberrations (other than dicentrics and rings), showed
a dose-dependent increase in the miners up to an
estimated dose of 3000 WLM. Finally, in the most
highly exposed group of miners (> 3000 WLM), there
was a marked decrease in the prevalence of dicentrics
and rings, relative to other exposed groups.

6. Chromosome aberrations in lymphocytes of workers
with internal depositions of plutonium

244. In another study, Brandom et al. [B67] conducted
chromosome analyses of peripheral blood lymphocytes
of 343 workers from the United States Department of
Energy facility at Rocky Flats, Colorado, and 68 non-
exposed controls from Rocky Flats and the Greater
Denver area. The radiation dose to the workers derives
mainly from internal depositions of a mixture of
239PuO2 (93–94%), 240Pu (6%) and 241Pu (0.5%). Physical
dose estimates of incorporated plutonium were derived
by two methods, urine assay ("systemic burden") and
lung counters ("lung burden"). External doses (x,
gamma rays and neutrons) were estimated with x-ray
film badges until 1970 and from 1970–1979, by thermo-
oluminescent badges. Although plutonium enters the
body primarily through inhalation, because of its differ-
ential organ distribution, the investigators did not have
any individuals with only lung burdens. There were
workers with estimates of solely systemic burdens and
those with both systemic and lung burden estimates.
The systemic burden estimates ranged from about
37 Bq to 370 Bq in the different workers with only
systemic burdens and from about 37 Bq to more than
1.5 kBq in those with both systemic and lung burdens.
The average external radiation estimates varied from
0.002 to 0.02 Sv for the different individuals and the
average years of exposure from 11.8 to 15.2; the mean
cumulative Sv value in each exposure group varied
from 0.03 to 0.33 Sv.

245. The individuals were classified into a number of
subgroups based on estimated exposures. Approx-
imately 100 cells were analysed for each subject. The
chromosomes were trypsin-banded and C-banded for
caryotypic examinations and all chromosomes in every
cell were analysed for numerical and structural aber-
trations. The data were categorized into: complex aber-
trations (dicentrics + rings + inversions + transloca-
tions); and total aberrations (complex aberrations +
deletions).

246. In the group with only systemic burden
estimates, there was no measurable increase in the
frequency of aberrations relative to controls. The
authors point out however that caution must be
exercised in interpreting these data since the dose
estimates are subject to some uncertainty; conse-
sequently, the lack of significant increase in aberration
frequency may be due to limitations in the sensitivity or
accuracy of the urine assay for systemic burden
estimates, or may reflect a true biological indication of
no response at these relatively low dose estimates.

247. In the group with both systemic and lung burden
estimates, there were significant increases in aberration
frequencies from the least to the most highly exposed
persons, in both the complex aberrations and total
aberrations (up from 0.5 per 100 cells in controls to
about 3.5 per 100 cells, complex aberrations; up from
about 1 per 100 cells in controls to about 5 per 100 cells,
"total" aberrations). The frequencies of deletions
strongly contributed to the total yield of aberrations,
especially in the lowest estimated exposure group.

248. When the data were appropriately pooled based
on total burden estimates and re-analysed, there was
again, a significant increase in the incidence of complex
and "total" aberrations with increasing dose. While the
increase in aberration frequency with dose was statisti-
cally significant (P < 0.01), it was not marked, rising
from an average of about 1% of cells with some
category of structural aberrations among the controls to
about 3% of cells affected in the highest exposure

249. Stepwise linear regression analyses demonstrated
that lung burden estimates, cumulative external irradi-
ation and age entered as significant independent
variables. The relative contribution of cumulative doses
of external irradiation to the aberration yields cannot
be accurately estimated. For instance, the frequency of
aberrations in the categories "complex" and "total" was
markedly higher in a group with an estimated external
dose of 0.15 Sv than in another with more than twice
(0.33 Sv) that dose. The mean plutonium burden
estimate for the first group was 4.4 kBq and for the
second, 1.3 kBq. It would thus seem that the difference
in aberration yields noted above is probably primarily
due to the amount of internal plutonium.