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NOTE

Throughout the present report and the annexes thereto, references to the annexes are indicated by a letter followed by a number: the letter denotes the relevant annex and the number the paragraph therein. Within each annex, references to its scientific bibliography are indicated by numbers.

Symbols of United Nations documents are composed of capital letters combined with figures. Mention of such a symbol indicates a reference to a United Nations document.

ANNEX A

DEFINITIONS OF QUANTITIES, UNITS AND SYMBOLS

1. The Committee has used in the present report the radiological quantities and units defined in the 1959 report of the International Commission on Radiological Units and Measurements (ICRU),[1] the relevant part of which is reproduced below.* It should however be noted that ICRU has appointed an *ad hoc* committee to examine the quantities and definitions of units and some modifications of existing definitions may shortly be recommended.

"1.1. *Absorbed dose* of any ionizing radiation is the energy imparted to matter by ionizing particles per unit mass of irradiated material at the place of interest.

"1.2 The unit of absorbed dose is the *rad*. One rad is 100 ergs/g.

"1.3. *Integral absorbed dose* in a certain region is the energy imparted to matter by ionizing particles in that region.

"1.4. The unit of integral absorbed dose is the *gram rad*. One gram rad is 100 ergs.

"1.5. *Absorbed dose rate* is the absorbed dose per unit time.

"1.6. The unit of absorbed dose rate is the *rad per unit time*.

"1.7. *Exposure dose of X- or gamma radiation* at a certain place is a measure of the radiation that is based upon its ability to produce ionization.

"1.8. The unit of exposure dose of X- or gamma radiation is the *roentgen* (r). One roentgen is an exposure dose of X- or gamma radiation such that the associated corpuscular emission per 0.001293 g of air produces, in air, ions carrying 1 electrostatic unit of quantity of electricity of either sign.

"1.9. *Exposure dose rate* is the exposure dose per unit time.

"1.10. The unit of exposure dose rate is the *roentgen per unit time*.

"1.11. *Intensity of radiation* (radiant energy flux density) at a given place is the energy per unit time entering a small sphere centered at that place per unit cross-sectional area of the sphere.

"1.12. The unit of intensity of radiation may be *erg per square centimeter second*, or *watt per square centimeter*.

"1.13. The unit of quantity of radio-active material, evaluated according to its radio-activity, is the *curie* (c). One curie is a quantity of radio-active nuclide in which the number of disintegrations per second is 3.700×10^{10}.

"1.14. *Specific gamma-ray emission* (specific gamma-ray output) of a radio-active nuclide is the exposure dose rate produced by the unfiltered gamma rays from a point source of a defined quantity of that nuclide at a defined distance.

"1.15 The unit of specific gamma-ray emission is the *roentgen per millicurie hour* (r/mch) *at 1 cm*.

"1.16. *Linear energy transfer* (LET) is the linear-rate of loss of energy (locally absorbed) by an ionizing particle traversing a material medium.

"1.17. Linear energy transfer may be conveniently expressed in *kilo electron volts per micron* (kev/μ).

"1.18. *Mass stopping power* is the loss of energy per unit mass per unit area by an ionizing particle traversing a material medium.

"1.19. Mass stopping power may be conveniently expressed in *kilo electron volts per milligram per square centimeter* (kev cm^2/mg)."

2. According to ICRU:[1]

"The absorbed dose, D (in rads), of any radiation must be multiplied by an agreed factor, RBE (relative biological effectiveness), whose values for different radiations are laid down by the International Commission on Radiological Protection (ICRP). This product, called the RBE dose, is expressed in rems where

$$\text{RBE dose (in rems)} = (\text{RBE})\ (\text{D})$$

"In the case of mixed radiations the total RBE dose is assumed to be equal to the sum of the products of the absorbed dose of each radiation and its RBE.

"RBE dose (in rems) = Σ [(absorbed dose in rads) (RBE)]."

For the sake of simplicity in the present report 1 roentgen of X-, beta or gamma radiation is assumed to correspond to a tissue dose of 1 rad and, since the RBE of these radiations is conventionally unity, the tissue dose may also be expressed as 1 rem.

3. The RBE values that have been used in the present report are those established by ICRP in establishing protection standards. The table below gives the values of RBE for different types of radiation.[2] The ICRP Committee on RBE is currently examining the concept and use of RBE in radiation protection calculations and new recommendations may shortly be made.

* The following is quoted from the above-mentioned ICRU report:

"*Symbols and nomenclature.* There are numerous national and international bodies that have reached varying degrees of acceptance of the use of symbols and units for physical quantities. However, there is no universal acceptance of any one set of recommendations. It is suggested that each country modify the symbols used herein, in accordance with its own practices. Thus one may write: kev, keV, or Kev; ^{14}C or C^{14}; rad per unit time, rad per time, or rad divided by time; rad/sec, rad/s, or rad.s^{-1}; etc. The most generally accepted system of symbols and units may be that contained in document UIP 6 (1956) prepared by the International Union of Pure and Applied Physics. These are in fairly close agreement with the recommendations of the International Standardization Organization project ISO/TC 12, the Conférence Générale de Poids et Mesures, Union Internationale de Chimie Pure et Appliquée, and the International Electrotechnical Committee."

TABLE I. RBE VALUES

1. *X-rays, electrons and positrons of any specific ionization*
RBE = 1
2. *Heavy ionizing particles*

Average specific ionization (ion pairs per micron of water)	*RBE*	*Average linear energy transfer to water (kev per micron)*
100 or less	1	3.5 or less
100 to 200	1 to 2	3.5 to 7.0
200 to 650	2 to 5	7.0 to 23
650 to 1,500	5 to 10	23 to 53
1,500 to 5,000	10 to 20	53 to 175

For practical purposes, an RBE of 10 is applicable to fast neutrons and protons up to 10 MeV and an RBE of 20 to heavy recoil nuclei for whole-body irradiation and the most sensitive critical organs.

REFERENCES

1. Report of the International Commission on Radiological Units and Measurements (ICRU) (1959). National Bureau of Standards, Handbook 78.
2. Recommendations of the International Commission on Radiological Protection. Brit. J. Radiol. Suppl. No. 6 (1955).

back to first page

ANNEX B

FUNDAMENTAL RADIO-BIOLOGY

CONTENTS

	Paragraphs
I. Introduction	1-3
II. Interaction between ionizing radiation and living matter	4-9
Energy dissipation by X- and gamma-rays and by corpuscular radiations	6
Ionization density—LET	7-8
Transport of energy	9
III. Quantitative aspects of radiation effects	10-35
Hit principle (target theory) and dose-effect relationships	12-18
The threshold problem	19-22
Direct and indirect effects of radiation	23-26
Influence of dose-rate and dose fractionation	27-30
Relative biological effectiveness	31-35
IV. Radiation chemistry	36-93
Water and aqueous solutions of simple compounds	38-49
Nucleic acids	50-61
Proteins	62-78
Polysaccharides	79-82
Macromolecular complexes	83-88
Detection of free radicals in whole cells by electron spin resonance (ESR)	89-93
V. Chemical factors modifying radiation response in cells	94-110
Oxygen effect	94-96
Effect of gases other than oxygen	97
Effects of gases under pressure	98
Hydration	99-100
Peroxide after-effects	101-102
Chemical protection	103-110
VI. Effect of radiation on cellular structures and their function	111-177
DNA synthesis	116-132
RNA and protein synthesis	133-157
Effects of radiation on antibody synthesis	158-160
General considerations of radiation effects in cellular metabolism	161-167
Effects of radiation on integrated functions	168-171
Growth rate	169
Mitotic delay	170-171
Inhibition of mitosis and cellular death: reproductive and interphase death	172-177
VII. Biological variables influencing radiation response	178-185
Concept of radio-sensitivity	178
Variations in radio-sensitivity with stage of division	179-181
Variation of radio-sensitivity with ploidy	182-184
Genetic control of radio-sensitivity in bacteria	185
VIII. Primary genetic effects of radiation	186-225
The genetic material	187-189
Point mutation	190-192
Radiation-induced mutagenic effects	193-194
Oxygen effect	195-196
Chemically-induced mutagenesis	197
Uptake of abnormal precursors	198-199
Comparison between various mutagenic agents	200
Biochemical aspects of mutation processes	201-204
Mutation expression	205-206
Chromosome breaks	207-210
Factors influencing the production of chromosome breaks	211-213
Genetic effects of incorporated radio-active substances	214-215
Possibility of transmutation effect with C^{14}	216-217
Local consequences of transmutation	218-222
Ionization dose per transmutation under uniform contamination	223-225
IX. Recovery at the cellular level	226-243
X. General conclusions	244-250
References	

I. Introduction

1. The effects of radiation on living matter must be envisaged at different levels of organization, those of individual molecules and macromolecules, subcellular structures, whole cells, tissues and organs, whole organisms, and populations of organisms. To understand the action of radiation, each system must be studied independently and in its natural context. The actions become more complicated as the organization level rises. At each level and for each effect studied, it is sometimes helpful to think in terms of the sensitive molecule or structure, the sensitive cell, tissue, or organ.

2. The present annex deals chiefly with macromolecules, subcellular structures, or isolated cells and cell populations. Our knowledge of the molecular organization of various cell organelles is increasing rapidly and the impact of molecular biophysics on fundamental radiobiology is greater than in the past. The molecular approach will eventually enable us to understand the effects of radiation on the impairment of fundamental processes in the cell. The effects of radiation on macromolecules or subcellular structures are thus of great importance in fundamental radio-biology.

3. This annex deals essentially with ionizing radiation; investigations with non-ionizing radiations are referred to only in so far as they bear on our understanding of the effects of ionizing radiations.

II. Interaction between ionizing radiation and living matter

4. The absorption of ionizing radiation by matter is followed by a complex of events the nature of which

depends on absorbed dose and the chemical and physicochemical composition of the irradiated material. Various stages can be recognized in the development of radiation effects. These are not sharply demarcated but blend into each other. Distinctions have some value, however, because they permit a partial analysis of the temporal sequence of events.

5. (a) *Elementary reactions.* These occur in a very short period of time, $\sim 10^{-17}$-10^{-15} seconds. They are primarily physical and result from the interaction between photons or ionizing particles and atoms and molecules. These interactions give rise to excitations and ionizations. Excited and ionized atoms and molecules are highly unstable and chemically active; rearrangements in the electron configuration of the excited structures lead to the primary products of radiation action which may be stable or unstable molecules, or free radicals.

(b) *Primary reactions.* Radicals and excited molecules formed as the result of elementary processes react chemically with neighbouring molecules and between themselves. This stage, the chemical stage, may last from a fraction of a second to hours.

(c) *Secondary reactions.* Elementary and primary reactions give rise to secondary reactions in which macromolecules of essential biological significance and major metabolic pathways are affected. Secondary reactions result, therefore, in alterations and impairment of cellular structures and functions, and may lead to biologically observable radiation injury. This, the biological stage, may last from a few hours up to years in long-lived multicellular organisms.

Energy dissipation by X- and gamma-rays and by corpuscular radiations

6. The elementary characteristics of ionizing radiation and the way energy is absorbed by ionization have been described in chapter II. Only part of the energy absorbed by an irradiated tissue gives rise to ionizations; the remainder, in a process called excitation, raises electrons of atoms or molecules to a higher energy level without expelling them. In its chemical or biological action, the energy absorbed in the excitation process is not considered to be as important as that absorbed in the ionization process. However information is incomplete on this point.

Ionization density—LET

7. In any interaction of ionizing radiation with matter, the ultimate transfer of energy is carried out by a charged particle. The rate of loss of energy by a particle along its path is proportional to the square of charge and inversely proportional to velocity. Hence, for any particle, the rate of loss of energy is greatest near the end of its track. Linear energy transfer (LET) is defined as the linear rate of loss of energy (locally absorbed) and is usually measured in keV/μ.

8. At a given dose the biological effect may vary considerably with LET; it may increase or decrease depending on the object irradiated and the effect measured. There is as yet no complete theory on the influence of LET (paras. 31-35).

Transport of energy

9. Free radicals, whose intrinsic lifetime is indefinite, usually disappear quickly because of their reactivity. Excited molecules have, in general, only a transitory existence in condensed systems since they are inherently unstable. Although excitation can lead to dissociation of the molecule, it is less likely to do so in the case of more complex molecules where excess energy can be distributed over many bonds. Energy degradation within the same molecule is known as internal conversion. Through internal conversion, the excited molecule is degraded in energy from a higher to a lower excited state, or returns to the ground state; the excess energy is converted into vibrational and rotational energy and may be transferred to other molecules. Energy can also be transferred from one molecule to another through processes known as exciton interaction and resonance transfer.[1] The increasing emphasis on the mechanisms by which energy migrates and on their role in radiation effects is reflected in recent symposia and reviews.[2-4]

III. Quantitative aspects of radiation effects

10. Known dose-effect relationships may be described under a limited number of headings. Their graphic presentation is often simple, linear in a few instances, and in general exponential or sigmoid. Thus, oxidation of ferrous ions and reduction of ceric ions in aqueous solution is, in certain circumstances, directly proportional to dose. These effects may be interpreted as due to radicals induced in the aqueous medium. However, in somewhat more complicated situations, e.g. the inactivation of enzymes in solution or in the solid state, there may be an exponential relationship between remaining activity and radiation dose. This relationship expresses, in part, the fact that inactivated molecules are still able to capture radicals and thus to decrease the number of radicals for inactivation of still intact molecules.

11. Even for complex systems like living cells, the experimental relationship between dose and effect is often a simple one. In the study of these relationships it is essential to define the effect clearly. For isolated cells, reproductive ability has been used most frequently as the criterion of damage. Cells which have lost reproductive integrity may still divide a few times. However, cells affected in this way can sometimes maintain the ability to accomplish for a certain time some metabolic or physiological functions at near normal rates, e.g., respiration,[5,6] protein synthesis,[7] motility.[8] The doses required for impairment of such metabolic functions are usually much greater than those necessary to impair reproduction.

Hit principle (target theory) and dose-effect relationships

12. According to the hit theory,[9-11] the biological effects of ionizing radiation on cells are due to hits in a sensitive component of the cell; hits produced outside this "target" are ineffective. Although, as originally formulated, the hit was considered to be an ionization or excitation produced directly in the target, the theory has been enlarged to include hits produced by diffusible products involved in indirect action.[12]

13. If a cell is inactivated by a single hit in a target or in any of a number of targets, it can easily be shown that the survival curve is exponential. The number of cells escaping biological modification (N) is then related to dose according to the formula $N = N_o e^{-aD}$, where N_o is the number of cells originally present, D is dose, and

α is a constant expressing the sensitivity of the cells. From this formula it follows that the number of survivors will be $N/N_0 = e^{-1} \sim 0.37$ for the dose $1/\alpha$ which is the dose that brings about one hit per target on the average. This 37 per cent dose is important in calculations of the volume of the target.

14. When two or more hits are necessary to destroy one target or when two or more targets in one cell have to be hit before the damage shows, the survival curves are no longer exponential but are sigmoid and have an initial shoulder when the logarithm of the survival is plotted against dose. In the latter case (two or more targets), the number of targets can be estimated from the survival curve by extrapolating the linear part of the semi-log-plot to zero dose. The value (greater than one) thus obtained on the survival axis is equal to the number of targets.

15. As a rule, with high LET radiations and neutrons, and in certain cases with X- or gamma-rays, exponential survival curves are observed for the inactivation of viruses and micro-organisms.[9] When the fraction of cells or subcellular structures affected is small, the number of responses is approximately proportional to dose. This has been found for the induction of mutations in bacteria, *Drosophila*, and other organisms; the mechanism seems to be one hit.

16. X-irradiated polyploid yeast cells[13,14] and isolated mammalian cells[15] have sigmoid dose-effect curves. The type of curve often depends on the LET of the radiation. Higher LET values may result in exponential survival for cells having sigmoid type curves for low LET radiations.[16]

17. Sigmoid survival curves are also expected when a population of individuals is irradiated, the susceptibility of which obeys certain distribution patterns.

18. Both exponential and sigmoid survival curves may have breaks (resistant tails). The interpretation usually offered is that the population studied contains a subgroup which is more resistant to radiation. In general there are two ways in which this could occur:

(a) The heterogeneity may be genetic; the more resistant individuals are mutants of the more sensitive. This situation can be recognized by isolating a clone from cells surviving higher doses and by establishing a new survival curve with the population from this resistant clone. The slope found corresponds to the slope of the resistant tail in the original curve. However, in some cases, attempts to do this have failed. With the widely used strain *E. coli* B, the rate of mutation to resistance is only about 10^{-5} per bacterium per generation and therefore probably too low to account for the appearance of the tail.[17]

(b) The heterogeneity may be physiological; in this case, if cells surviving at the higher doses are isolated, the survival curve of the new population shows the same resistant tail as the original one. This holds in haploid yeasts where budding cells appear to be more resistant.[18] There is similar phenomenon with *Pneumococcus* transforming principle.[19] A resistant tail may also be seen with a bacterial population containing cells in both the logarithmic and stationary phase of growth; the logarithmic phase is more radio-sensitive.[20,21]

The threshold problem

19. The observation of an exponential survival curve may be interpreted as a one-hit process. The same applies to the linear relationship for mutation induction when the number of mutations is small compared to the number of loci at risk; any dose, however small, has a probability of producing the effect.

20. Sigmoidal survival curves may be interpreted as an indication that inactivation results from multiple hits in a single target or inactivation of multiple targets by one or more hits in each. There is also a finite probability that any dose may produce an effect. Thus the existence of biological responses with sigmoidal dose-effect curves do not necessarily prove the existence of a threshold dose.

21. Even if recovery processes occur at the cellular level, these conclusions remain valid; such recovery merely changes the slope of the dose-effect curve.

22. Without extensive empirical data and detailed knowledge of the various steps between initial absorption of radiation and expression of biological effects, discussion of the threshold question is largely limited to theoretical considerations. In the only instance in which it has been possible to obtain unequivocal experimental data, the induction of phage growth in lysogenic bacteria, no threshold was found; one ion pair per cell was effective.[22] It is therefore prudent to assume, as in the last report of the Committee, that "biological effects will follow irradiation, however small its amount".[23]

Direct and indirect effects of radiation

23. Of the models proposed to explain observed dose-effect relationships, the simplest is the target theory based on the assumption that inactivation is caused only by ionizations *inside* the target—"direct action".

24. Although the concept of a "target" has been maintained in most theories, it has become increasingly apparent that at least part of the biological effect is due to chemical events outside the target. In this event damage to the target is secondary—"indirect action".[16,24]

25. As yet there is no general agreement on the relative importance of direct and indirect action in living cells. The modification of damage by oxygen or chemical protective agents has sometimes been interpreted as evidence that indirect action is predominant. It has however been shown that the effect of oxygen and some protectants is also consistent with direct action, if it is assumed that the effect of radiation on the target is a two-stage one.[25,26] The primary event might then be partly or totally reversible.

26. The problem of direct versus indirect effects of radiation has been comprehensively reviewed by Timofeev Ressovski and Rompe[2] with an analysis of mechanisms of energy migration and transfer in the heterologous system. Their theory allows for chance fluctuations in the occurrence of both direct and indirect effects, and for the mechanisms of propagation of radiation injury in time and space. Depending on the structure or function damaged, either direct or indirect effects may be considered predominant.

Influence of dose-rate and dose fractionation

27. Variation of the irradiation rate (fractionation of dose or variation of dose-rate) may influence the biological effect in some instances. When radiation damage is irreparable, no modification of the response is expected; if a modification is seen, it is generally assumed to repre-

sent a repair mechanism. Mice, *Drosophila,* plants, and several other species (C, table VII) have been extensively studied. Other examples are *Arbacia* eggs[27] and mammalian tissue culture cells. In *Arbacia* sperm, however, no repair has been observed.[28,29]

28. If the phenomenon under study is single hit, e.g. induction of point mutations, repair processes would reduce the magnitude of the slope of the dose-effect curve. Russell[30] discovered that low dose-rates were less efficient than high dose-rates in inducing mutations in mouse gonial cells. This dose-rate effect was maximal at 0.82 r/min; further reduction of the dose-rate had no further effect on mutation rates.[31] Russell's finding, which stimulated similar studies by others, has been confirmed in several species. Low dose-rates also greatly diminish the sterilizing effects of radiation in female mice and increase survival of spermatogonia.[39]

29. The effectiveness of fractionated doses to the mouse testes has been demonstrated with doses in the range of 1600 rad.[40] In experiments with *Drosophila* at low doses and different stages of spermatogenesis, no effect of dose fractionation has been observed.[32]

30. The effect of dose-rate on multi-hit processes is not difficult to explain. If the rate of delivery is reduced so as to increase the time between two successive events (hits) significantly, and if the individual lesions due to hits can be repaired within a certain time, lowering the dose rate or fractionation of the dose will result in a diminished frequency of effects for a given total dose. The role of chromosome aberrations may be of particular importance in monkey or human embryonic tissue cultures. Some reports indicate that these tissues are two or three times more sensitive than those of mice.[33,34] Investigations of the repair of pre-mutational damage have been carried out with many species, including mammals[35,36] insects[37,38] and plants.[264,516] This subject is discussed more fully in annex C.

Relative biological effectiveness

31. With radiations of different quality, the absorbed doses required for a given effect are usually not the same for different types of radiation. The extent to which radiations of different quality differ from each other in this respect is a measure of their relative biological effectiveness (RBE). The RBE of two radiations is defined as the inverse ratio of the respective doses that are necessary to bring about a given effect. The radiation standard chosen by the ICRU is an X- or gamma-radiation having a LET in water of 3 keV/μ delivered at a rate of about 10 rad/min.

32. In the simplest cases, the mechanism underlying the difference in efficiencies of radiations can easily be explained. For an event which is inhibited by the absorption of a minimal amount of energy, such as the inactivation of an enzyme or virus, the low ion density radiation will be more effective than high ion density, because some of the latter ionizations will be wasted. On the other hand, radiation with a high density of ionization will be more effective when larger amounts of energy are needed (simultaneously or within a relatively short time or within a certain volume) to produce the effect in the sensitive structure.

33. Thus, RBE depends not only on the LET of a given radiation but also on the effect studied, and this dependence may assume various forms. Thus, Zirkle[41] has pointed out that there are experimental situations in which RBE and LET are directly related, inversely related, in which RBE shows a maximum for a certain value of LET, and in which RBE is constant. Other factors make the picture even more complex; RBE values may depend on dose, dose-rate, presence of oxygen, and physiological conditions.

34. The LET concept itself is complex. The kinetic energy loss of a particle is discontinuous and subject to statistical fluctuations.[42] Furthermore, it varies along the track. For these reasons an average value must be calculated. In principle, however, RBE not only depends on this average value of LET, but also on LET distribution. The following figure[43] attempts to summarize experimental data on bacterial, plant and animal cells.

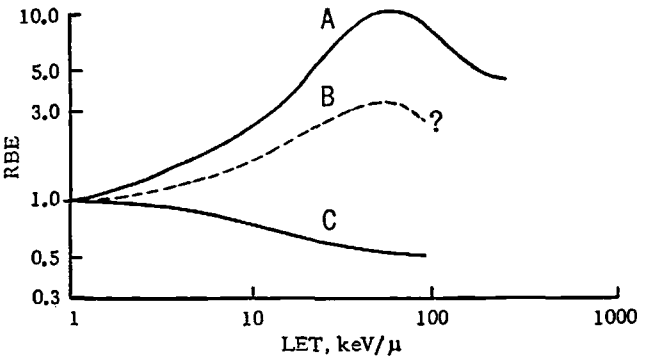

Figure 1. Variation in RBE with LET, for biological materials irradiated in aerobic conditions[43]
A: plant cells[577–582]
B: animal cells[203, 577, 583–585]
C: two strains of bacteria[183, 586–587]

35. To assess the RBE of a certain radiation, dose-response curves of the particular biological effect are determined for both test and standard radiations. If both curves coincide when all dose values of the test radiation are multiplied by a constant factor, the RBE is equal to this factor. Sometimes the curves do not have identical shapes; the RBE value then depends on dose. This comparison pertains to absorbed dose. If this dose is not uniform throughout, the average value is used. This may not be strictly correct if the biological effect depends on dose. There are many other complications that make experimental RBE values difficult to interpret. The values are, however, useful in the practice of health physics, where upper limiting values of RBE are used to transform dosages measured in rad to rem.

IV. Radiation chemistry

36. Since water constitutes 70 per cent or more of cell mass, water molecules take up most of the energy imparted to cells by ionizing radiation and may be important in the damage to vital cell components. Knowledge collected during the last decade about the chemical changes induced by irradiation of water and aqueous solutions of simple compounds is therefore of great importance to radio-biology. Work has been done on the radiation chemistry of solutions of nucleic acids and other macromolecules to gain some insight into the mechanism by which reactive intermediates generated in water attack these molecules. The main results from those fields of research will therefore be summarized in this chapter.

37. In interpreting these results, it is generally assumed that free radicals are important in the chemical

reactions resulting from ionization and perhaps from excitation of water molecules. At present, there is abundant evidence to support such a view. Recently, development of the electron spin resonance technique has provided a method for direct study of free radical formation in certain irradiated materials.

Water and aqueous solutions of simple compounds

38. Most reactions in irradiated water can be explained satisfactorily by assuming the formation of H° and OH° radicals. Recent reviews[44-46] of the chemical effects of ionizing radiation have shown the usefulness of the radical hypothesis in interpreting the rapidly growing body of experimental data, although some uncertainty still exists with regard to the H° radicals and their distribution around the track of an ionizing particle. It might be that what has been called an "H° radical" is in reality a hydrated electron, H_2O^-.

39. For each 100 eV of dissipated energy some 4 H_2O molecules are split into OH° and H°. OH° radicals can combine to H_2O_2 and H° radicals to H_2. A considerable fraction of the radicals react in this way to give "molecular products" before there is any significant diffusion or reaction with solute molecules. In chemically pure water, however, only very small amounts of molecular products can be detected, because they are reverted to water molecules through back reactions with free H° and OH° radicals.

40. When solutes capable of reacting with H° or OH° radicals, thereby preventing the back reaction, are present, the products H_2O_2 and H_2 are produced in measurable amounts. Their yields depend on LET, a greater LET giving rise to a larger amount of molecular products through combination of free radicals. The molecular yield also depends on the efficiency with which free radicals are scavenged by solute molecules. Some very efficient scavenging solutes can depress the formation of H_2 and H_2O_2 considerably.

41. A very common solute is O_2. It reacts with H° radicals to give the radical $O_2H°$. This explains why the yield of various radiation-induced chemical reactions is dependent on the presence of O_2. The $O_2H°$ radical is more stable than H° and OH°. When no solutes other than O_2 are present, most $O_2H°$ radicals will combine according to the reaction $2\ O_2H° \rightarrow H_2O_2 + O_2$.

42. The primary products in irradiated water may have oxydizing or reducing properties depending on the redox potential of the solute concerned, on the qualities of other solutes (e.g. O_2, which converts reducing H° radicals to $O_2H°$ radicals which may have oxydizing action), or on pH.

43. The influence of pH is explained by the following ionic equilibria: $H° + H^+ \leftrightarrows H_2^+$; $OH° \leftrightarrows H^+ + O^-$ and $O_2H° \leftrightarrows H^+ + O_2^-$. It should be noted that, in neutral solutions, $O_2H°$ radicals have far less oxydizing power than at low pH's. The oxygen effect in living systems can therefore probably not be interpreted as an enhancement of oxydation through the reaction $H° + O_2 \rightarrow O_2H°$.

44. Application of these data to radio-biological systems is by no means straightforward. In the first place, the diffusion range of free radicals in living cells is very limited,[47] because many molecules can react with free radicals, thereby protecting more vital components. Cell structures can be attacked, therefore, only by radicals formed in close proximity, and the damage to certain molecules will be much less in cells than in dilute solutions. Secondly, the presence of great numbers of simple and complex molecules in cells may give rise to secondary and tertiary reactions which differ from those in simple solutions.

45. Knowledge of the primary reactions in irradiated water has been derived largely from the study of aqueous inorganic solutions. Much experimental work has also been done on aqueous solutions of organic compounds. However, for many changes in solutions of simple molecules, the reaction mechanism has not been unambiguously established.

46. There is evidence for the formation of hydroperoxides in the presence of oxygen:

$$RH + OH° \longrightarrow R° + H_2O$$
$$R° + O_2 \longrightarrow RO_2°$$

$$RO_2° \xrightarrow{\text{reduction}} RO_2^-$$
$$RO_2^- + H^+ \longrightarrow RO_2H$$

47. In some instances hydroperoxides are believed to be labile intermediates, but stable peroxides have also been found, e.g. after irradiation of solutions of various amino acids and of pyrimidine bases[48] and their nucleosides and nucleotides.[49,50] The formation of hydroperoxides may enhance oxydation, e.g. increase oxydation of ferrous ions in acid solution where there are organic impurities. This can be prevented by addition of Cl^- ions; these react with OH° radicals ($OH° + Cl^- \rightarrow OH^- + Cl°$) and thus modify the sensitizing action of organic molecules.[51]

48. Reactions between radicals and oxygen, and between radicals and hydrogen-atom-donating compounds, have been shown to be important biologically. In the bacterial spore, radicals formed that are biologically effective if they react with oxygen may be removed by hydrogen donators such as H_2S prior to O_2 reaction.[52,53] Such mechanisms have been proposed for other systems.[49,54,55]

49. Further chemical reactions which may bear on radio-biology are the oxydative deamination of amino-acids,[56] the decarboxylation of organic acids,[45] the oxydation of SH-compounds to the -S-S- dimer,[57] and the decomposition of glucose by ionizing radiation.[58]

Nucleic acids

50. Irradiation of nucleic acids in aqueous solutions leads to several different chemical changes which affect both the purine and pyrimidine moieties and the sugar-phosphate backbone. As yet, it is impossible to give a consistent and quantitative description of these chemical effects of irradiation. Because of the diversity and complexity of the chemical changes, only the main pathways are considered to be established.

51. The chemical changes produced by irradiation of dilute solutions of nucleic acids are, for the most part, initiated by radicals formed in the aqueous media. In agreement with results of experiments with simple nucleic acid components, there are two main reaction pathways by which radicals attack nucleic acids in aqueous solutions: (*a*) destruction of the bases, the predominant site of chemical attack, and (*b*) oxidation of the sugar moiety.[58-61] The products of irradiation of the bases in the presence of oxygen differ from

those formed in its absence. In oxygen-free solutions, pyrimidines are converted into products of undetermined structure, without any specific ultra-violet absorption.[61] Some guanine residues are converted to 2:4-diamino -5-formamido-6-hydroxy-pyrimidine which is attached to the sugar by a labellized glycosidic linkage; from this they are gradually released as free bases. It is believed that the attack on adenine forms the corresponding formamido-pyrimidines, although this has not been directly demonstrated in irradiated DNA.[59] The yield of chemically altered bases is highest for pyrimidine residues and lowest for purine residues,[59,61-63] a circumstance which reflects their comparative radio-sensitivity.

52. In aerated solutions of nucleic acids, the hydroperoxides of pyrimidine bases are formed with the saturation of 5, 6 double bonds, and under oxygen this reaction becomes the dominant one.[61] In DNA, only hydroxyperoxides of thymine are stable and only these remain attached to the sugar-phosphate backbone.[61] In the presence of oxygen the sensitivity of all bases in DNA solution is increased two to three times; under these conditions a presumed 80 per cent of radicals attacking DNA combine with the base components.

53. The attack of radicals on the sugar moiety leads to formation of labile phosphate esters. Evidence for this is seen in the large quantities of inorganic phosphate that can be liberated by the acidic hydrolysis of irradiated solutions.[72] It is believed that this results from oxidative formation of carbonyl groups in sugar moieties.[61] In addition to the formation of labile phosphate esters, the attack on the sugar component breaks phosphodiester bonds and liberates small amounts of inorganic phosphate.[60,64] From experiments with simple phosphate esters,[65] it appears that inorganic phosphate must come from end groups present in the intact molecule, having been formed during earlier stages of irradiation by main chain scissions.

54. The direct measurement, with prostate phosphomonoesterase, of the number of breaks induced in the sugar-phosphate backbone has revealed that the yield from this process is 20-25 per cent of the yield in terms of base destruction.[66] The same percentage is found if the release of free bases from irradiated DNA is used to measure the attack on the sugar-phosphate moiety.[59,62,67]

55. Studies of physicochemical changes in nucleic acids after irradiation are, so far, chiefly confined to deoxyribonucleic acids. In the double-stranded helical DNA molecule, both types of chemical lesions introduced by ionizing radiation, destruction of bases and breakage of phosphodiester bonds, must lead to an altered configuration in solution and consequently to changes in physicochemical properties. The destruction of the base results in local dissociation of the double-stranded structure, and the break in one of the chains results in increased flexibility; two independent breaks at approximately opposite positions in each of two intertwined chains lead to a scission of the whole molecule. There is much evidence supporting this general picture. Thus, the critical temperature for the thermal denaturation of irradiated DNA is reduced.[68] Likewise, the intrinsic viscosity of irradiated DNA solutions shows marked decreases that reflect coiling of the partially denatured molecule and a fall in molecular weight.[69-71] Further evidence for degradation is provided by light-scattering,[71,72] flow birefringence,[72,73] sedimentation and diffusion studies,[74,76] and chromatography on ecteola cellulose column.[77] The breakdown of some of the secondary hydrogen-bonded structure has been shown by the increase in ultra-violet absorption near 260 mμ after small doses of irradiation,[61,70,72] and also by titrimetric studies.[70,78]

56. Degradation of DNA proceeds for some time after irradiation, as judged by viscosimetric measurements.[76] This "after-effect" is more pronounced if DNA is irradiated in air-saturated solution.[79-81] There are several hypotheses to explain this kind of instability; the decay of some unstable pyrimidine hydroxyperoxides,[60,61,82] and hydrolysis of labile acyl-phosphates[61,64] are the most plausible of them.

57. In dilute solutions, the indirect action of radiation prevails. With increasing concentration of DNA the relative importance of this effect decreases in favour of the direct action. This has been shown in experiments in which damage to DNA, as a function of concentration, was studied in the presence of iodine ions which almost entirely prevent the indirect effects of radiation.[83-85] Thus, Mekshenkov ascertained that 0.1 per cent solutions of DNA are almost entirely protected against X-radiation by iodine ions (predominance of indirect effect). With increasing concentration of DNA, however, the protective ability of iodine ions decreases so that in a 20 per cent solution, 80 per cent of DNA molecules present are damaged[86,87] (predominance of direct effect).

58. DNA molecules irradiated in the dry state or in a slightly moist condition are damaged mainly by the direct action of ionizing radiation. With radiation doses of $\sim 10^6$ rad, in addition to the main chain scission, an intermolecular cross-linking takes place which leads to the appearance of branched molecules as judged by viscosity, sedimentation and light-scattering studies. With increasing doses up to 10^7 rad (the threshold dose depends on water content), this process renders DNA insoluble in water and gives rise to gel formation. Both processes proceed simultaneously, but their relative role in the damage depends on moisture content, presence of oxygen, and nature of ionizing particles.[68,71,88-90]

59. The rates of main chain scission and branching induced by electrons are about the same at moisture contents up to 25 per cent, and are largely unaffected by oxygen. With swollen DNA gels having a water content of 25 to 70 per cent, intermolecular cross-linking predominates over the scission of the main chain in the absence of oxygen. However, in the presence of oxygen, the ratio between the effectiveness of the two processes is reversed. Above 75 per cent water content, and even in the absence of oxygen, no gel is formed.[88,89] Alpha-particles are much less effective in the branching process than electrons. With alpha-particles only a limited amount of cross-linkage is found in the absence of oxygen, and this is independent of the moisture content. In the presence of oxygen one main chain break is produced by nearly every alpha-particle traversing a DNA molecule.[90]

60. It is believed that clusters of ionization are responsible for the main chain breaks; cross-links result from the combination of active points formed by ionization[89,90] for which carbon radicals are likely candidates. Some direct support for the formation of metastable species is provided by the observation of strong gamma-induced phosphorescence in frozen solutions of DNA and RNA.[91] With direct irradiation of dry DNA preparations by gamma-rays, the ESR method reveals the presence of one radical per 10^5 DNA molecules for a dose of 2×10^3 rad.[92]

61. It is worthwhile to mention that ultra-violet radiation also causes aggregation of DNA[94] and, to a lesser degree, of RNA in the dry state.[95,96] In water solution, irradiation of DNA with ultra-violet light induces covalent crosslinks.[96,97] The native secondary structure is almost preserved as shown by ultra-centrifugation in caesium chloride. These cross-link processes are probably connected with dimerization of thymine or uracil residues.[98,99]

Proteins

62. Changes in the structure of proteins irradiated in dilute aqueous solution are mainly attributable to attack by free radicals and other active species from water. In cells, free radicals account for ~ ½-¾ of the effect; in very dilute pure solutions they account for almost the entire effect.[100]

63. Thiol groups, when present, appear to be the most sensitive parts of proteins. These -SH groups become oxydized, as shown by titration,[101] thus creating new disulphide bonds with a G* value of about 3. The same process has been observed with enzymes,[102] although the high G value for the oxydation of those enzymes which depend on -SH groups for activity does not always correspond to the G value for inactivation.[103] Conversely, by other mechanisms, disulphide bonds can be reduced by irradiation, a process which leads to the formation of new thiol groups.[104,105]

64. Proteins, amino acids, and peptides, in solution, can liberate ammonia on irradiation with large doses, and can at the same time form carbonyl and amide compounds.[106,107] These products are formed in part from amino-groups and in part from peptide bonds. This reaction involves the formation of imino-groups as intermediates. The imino-groups are hydrolized, leading to the rupture of polypeptide chains.[102]

65. The effect on aromatic rings of amino acids in proteins resembles closely the effect on aromatic amino acids themselves. Changes in optical density in the UV absorbing region of some proteins, when irradiated, are similar to those produced in a tyrosine solution.[108] Similarly, a decrease in optical density at 280 mμ has been found for tryptophan itself[109] as well as for proteins rich in this amino acid.[110]

66. Protein peroxides have been detected after irradiation of proteins in oxygen-containing solutions.[111]

67. Model experiments with protein solutions have revealed that the latent damage, caused by radiation in myosin molecules responsible for the radiation after-effects, can be eliminated by formation of complex compounds with actin molecules if these are introduced into the solution immediately after irradiation.[112]

68. Long-lived activated states persist for a few days in protein molecules irradiated in aqueous solution. Activation is associated with disruption of the protein electron structure; this has been confirmed by the ESR method.[113,114] The ESR method has revealed prolonged retention, by protein molecules (myosin, pepsin), of unpaired electrons appearing after irradiation of protein solutions. A close relationship has been established between these electrons and radiation after-effects in the same system. When irradiated solutions are slightly warmed there is an accompanying "thermal effect", and unpaired electrons in the protein molecules disappear. This confirms the previous assumption that prolonged retention of unpaired electron-excited energy is a cause of radiation after-effects.[114]

69. Model experiments with irradiated myosin have revealed "oxygen effect" at the molecular level. Inactivation of myosin's ATP function by irradiation has two stages: first (without the involvement of oxygen) is the long-lived "excited" state of the protein molecule capable of interaction with molecular oxygen; its enzymatic activity is still preserved at this time. Inactivation occurs in the second stage as a result of interaction with oxygen. In an aqueous solution of myosin, "oxygen after-effects" constitute most of the total "oxygen effect".[113,115-117] These results from a molecular system correspond well with those from studies on a biological system and thus demonstrate the biological importance of these events. In dry spores of *B. megaterium*, oxygen interaction with radiation-induced states can be almost "immediate" as well as post-irradiation.[118] The radiation-induced species have proved to be free radicals in experiments involving post-irradiation heat, nitric oxide, and H_2S treatments,[119-123] coupled with physical experiments (paramagnetic spin resonance studies) of a similar kind.[82,124] In these experiments, as in those described above with myosin, most of the oxygen effect can occur for an appreciably long time after irradiation. Furthermore, an intermediate state (the metionic state), the consequence of the reaction of oxygen with radiation-induced active species, has been postulated from studies of another biological system.[125]

70. The damaging effects of heat and oxygen in the after-effect response of irradiated myosin solution have proved to be independent of one another. There are thus two distinct forms of latent damage in the same irradiated protein molecule; this agrees with the data of Gordy and his colleagues, who established, by ESR studies, the presence in irradiated protein molecules of two types of spectra—some modified by the action of oxygen and others insensitive to it.[126,127]

71. As a consequence of the chemical changes of proteins under irradiation, one can expect changes in physical-chemical properties. Changes in chromatographic,[128] absorptive,[129] and electrophoretic[130] properties have been seen.

72. In contrast to irradiation in the dry state, the molecular weight of proteins increases after irradiation in solution.[131-133] From chemical evidence there may be several reasons for this. Attack of the tyrosine moieties may induce polymerization as with tyrosine solutions[134] (melanin formation). In addition, disulphide linkages may be formed among protein molecules. Finally, a re-aggregation of broken molecules may take place, the molecules being held together by freshly formed hydrogen bonds.[135]

73. Irradiation of certain protein solutions (with doses up to 6×10^6 rad) does not lead to perceptible effects on physical, chemical and biological properties immediately after irradiation. However, exposure to heat,[136] urea,[137] or UV,[170] alters X-irradiated protein solutions (coagulation, denaturation) more than non-irradiated solutions.

74. In the case of catalase and trypsin inactivation, an after-effect has also been shown.[138,139] The extent of this depends very much upon the post-irradiation temperature to which the irradiated enzyme was exposed.[139]

* "G" represents the number of molecules changed or produced for each 100 eV of energy absorbed.

The presence of oxygen after irradiation appears to be, in general, unimportant; the after-effect may be attributable to the formation of protein peroxides, of thermolabile molecules, or to other causes.[140,141]

75. According to present knowledge, enzyme inactivation is attributable to the action of hydroxyl radicals.[142,143] This hypothesis is supported by the observation that iodine ions serve as protectors for catalase inactivation; it is to be expected that these ions react more readily with hydroxyl than with hydrogen radicals.[144]

76. Very little is known of the chemical changes in proteins brought about by irradiation in the dry state. The involvement of disulphide linkages has been demonstrated by the close resemblance between electron spin resonance spectra of a number of proteins and that of irradiated cystine,[127] and by the fact that irradiated ribonuclease, like ribonuclease with its S-S bonds reduced, can be digested by trypsin whereas the native protein is resistant.[145] A general increase in ultra-violet absorption,[135,146,147] accompanied sometimes by a shift in the position of the absorption maximum, indicates an attack on aromatic amino acids. Changes in content of other amino acids have also been demonstrated[147,148] and differences in sensitivity between particular amino acids have been noted.[147] The formation of ammonia and amines with the development of carbonyl and carboxylic end groups in the hydrolysates of irradiated proteins is attributable to an attack on amino acids side chains and on peptide bonds.[60] Susceptibility of peptide bonds to main chain scission is apparently rather low because no such breaks have been detected in serum albumin irradiated with doses up to 2.5×10^8 rad.[147] The oxygen effect observed upon irradiation of dry proteins seems to be connected not only with the excitation of protein molecules but also with the excitation of oxygen molecules which in turn act on hydrogen bonds within protein molecules.[117] The most typical changes in physical, chemical property are those changes which occur *in vivo:* isoelectric point, decrease in sedimentation coefficient, or aggregation as a result of hydrogen bond formation between molecules with disorganized secondary and tertiary structure.[133,135,147]

77. The important aim of studies of the action of ionizing radiation on proteins is to understand the mechanism of radiation-induced enzyme inactivation. The catalytic capability of an enzyme is determined, most probably, by an active site composed of only a very small number of amino acid residues maintained at the surface of the enzyme molecule by secondary and tertiary bonds. Thus, enzyme inactivation can be accomplished either by chemical alterations in the amino acid residues within an active site or by disruption of essential configuration.

78. The efficiency of inactivation through ionization is very high, with $G \sim 1$. This implies that one ionization or cluster of ionizations anywhere within or near a molecule inactivates that molecule. This makes the hypothesis of inactivation via an attack on the site of specific activity improbable. Consequently, inactivation of enzymes by radiation is discussed here in terms of disruption of the secondary and tertiary structure following the production of an electric charge inside the macro-molecule[149] and migration of the ionizing energy along the covalently bonded structure. Energy then becomes localized on weaker bonds,[150,151] particularly on S-S disulphide bridges responsible for maintaining the various chains of the enzyme in the native structure.

Polysaccharides

79. The most noticeable effect of radiation on polysaccharides is chain degradation. This holds for all conditions of radiation[152] as shown by decrease in viscosity, changes in light-scattering, electrophoretic and ultra-centrifuge patterns. The most probable mechanism of degradation is one involving free radicals formed from water, because Fenton's reagent, used as a source of free radicals, induces the same damage.[153]

80. New acid and aldehyde-reducing groups are formed in polysaccharides after irradiation.[107,154] Small fragments have been found, e.g. gluconic and glucoronic acids in the case of dextran. Mass spectrometry data demonstrate the formation of H_2, CO and CO_2 when dry cellulose is irradiated.

81. While the effects of irradiation on polysaccharides in solution and in dry state are much the same, cellulose and pectin, when irradiated in a dry state show an after-effect, but only if stored dry in the presence of oxygen.[155] This is probably due to long-lived radicals formed with oxygen. In addition to degradation, branching has been observed in the dry state.[156] The branches are random in length and spacing. All branch points are probably tetra-functional. Branching of polysaccharides in aqueous solutions has not been reported.

82. High molecular weight polysaccharides such as hyaluronic acid in solution (synovial fluid) are depolymerized[151] when irradiated with relatively low doses of X-rays (9,000 r), and the process continues about twenty-four hours after irradiation. Viscosity and light-scattering measurements have proved that, during the after-effect, depolymerization continues. The most probable sites of depolymerization are the -O-C-phospho-ester bonds. The addition of cysteamine[158] protects the synovial fluid, although in the absence of oxygen (presence of nitrogen) synovial fluid is more radiation-sensitive. A detailed study of ESR of irradiated polysaccharide has not thrown any light on the observed chemical changes. Internal crosslinking has been suggested[159] although direct proof, using hyaluronic acid, does not exist.

Macromolecular complexes

83. There is growing interest in relating the results obtained by irradiating isolated compounds of macromolecules in aqueous solution, and even in the pure solid state, to those from integrated macromolecular complexes (section VI below). Nucleoproteins are probably the closest models of nucleic acids as they exist in the cell, although the status of nucleoproteins *in vitro* may be very different from that *in vivo.*

84. Protein has a protective effect because it traps radicals that would otherwise reach the deoxyribonucleic acid (DNA), but the extent of this trapping is unknown.[160] However, some protective action of nucleic acids on the denaturation of ovalbumin as measured by the number of titrable sulfhydryl groups has been reported.[161]

85. Nucleoproteins from the same source but with different protein contents show different radio-sensitivities. Dilute solutions of DNA nucleoprotein with N/P ratio smaller than 2 are more radio-sensitive than DNA with N/P greater than 2. Radiation damage is established from a decrease in viscosity. These differences can be attributed to the influence of protein content on

the configurations of DNA in the complexes rather than to some protective action of protein.[162,163]

86. If there is a radio-lesion, several possible sites of disintegration and disruption of a nucleoprotein can be envisaged. These include bonds between nucleic acids and protein. Their response may explain why irradiated nucleoproteins do not swell in water as readily as unirradiated material, and why trypsin yields free DNA more quickly from irradiated nucleoproteins.[164]

87. On irradiation with electrons (2×10^4-2×10^6 rad), part of the DNA of sperm heads is cross-linked to form a loose gel-like network;[165] this does not appear to be due to secondary valence forces. Such cross-linkage has been postulated to be the cause of inactivation of bacteriophages by ionizing radiation.[165] This seems less plausible than the hypothesis that inactivation is due to production of carbon radicals in phage DNA. Such radicals may combine with oxygen, react with a hydrogen-atom donor, or become inactive by an unknown process if neither oxygen nor hydrogen is present.[166,167]

88. It is not yet clear which chemical changes are most important in the loss of biological activity of nucleic acids. No data clearly relate radio-sensitivity of biologically active nucleic acids to chemical changes produced by ionizing radiation. From studies on the inactivation of transforming DNA by ultra-violet radiation, by heat denaturation,[168,169] and by radio-mimetic substances,[170] damage to the bases seems important. On the other hand, a break in one of the chains of double-stranded DNA, or even scission of the whole molecule, does not necessarily lead to loss of activity. The molecular weight of the transforming DNA can be lowered approximately one order of magnitude by ultrasonic disruption without completely inactivating DNA.[171] The inactivation yields, from decay of P^{32} incorporated into single- and double-stranded DNA phages indicate that, whereas all breaks in single-stranded DNA inactivate the phage, both strands must be broken in double-stranded DNA phages, a fact which accounts for the lower efficiency (ca.10 per cent).[172]

Detection of free radicals in whole cells by electron spin resonance (ESR)

89. Although the radiation chemistry of water and of macromolecules *in vitro* can provide useful information on models of primary reactions *in vivo*, complete information depends on studies on the chemistry of the biological constituents after irradiation of living organisms. Progress in this field has been obtained recently with development of the electron spin resonance technique (ESR); this allows study of free radical formation in biological systems.[173]

90. Through this method, unpaired electrons have been detected in a variety of materials. When applied to detection of free radicals, the material irradiated must be stabilized to prevent diffusion of the radicals, e.g. measurement has to be carried out in solids, in frozen solutions and suspensions, or in dry biological material. In principle, quantitative estimates of the number of unpaired electrons in a sample are possible. In practice, it is difficult to attain reasonable accuracy.

91. Data derived from irradiated biological materials are not easily interpreted. They do not necessarily relate to those free radicals responsible for the biological effects of irradiation because many unpaired electrons arise in biologically less important molecules. From studies of simpler systems it is known that the presence of even slight amounts of impurities can modify the spectrum appreciably. It is not yet possible to identify those free radicals that give rise to the particular pattern of electron spin resonance absorption in irradiated biological material. Therefore, attempts have been undertaken to show a parallelism between radiation-induced ESR phenomena and biological effects on the same material.

92. In seeds of the grass *Agrostis stolonifera*, the effect of irradiation on growth inhibition decreases when water content increases. This has been related to the observation that the fraction of free radicals persisting for longer times after irradiation also decreases with increasing water content.[174] In seeds of *Vicia faba*, both the sensitivity and free radical concentration after irradiation decrease with increasing water content.[175] In barley seeds, studies have been made of the influence of water and LET on radicals detected by ESR techniques.[176] Attempts to relate biological and ESR results on dry pollen grains have been reported.[177] A parallel between biological end points and ESR data has been established in bacterial spores in studies of the effects of oxygen, heat, and NO treatments on the biological and physical responses.[52,53,118,124] The ESR method, applied to the investigation of lyophilized tissues of whole-body irradiated rats, also demonstrates the presence of stable radicals which vary with the different tissues. After irradiation with 1,000 rad the amplitude of the spectra does not change in any of the tissues with the exception of spleen where there is a sharp decrease immediately after irradiation.[178] The ESR method has also been used to study the effects of different gases[179] (air, N_2, NO) and of protective substances like cysteamine and AET on the production of free radicals.[180,181]

93. The results obtained so far through the ESR technique are summarized in the following propositions:[11]

"(*a*) Ionizing radiation produces free radicals in living material;

"(*b*) The concentration of free radicals produced by radiation increases with increasing doses;

"(*c*) The measurable concentration of free radicals depends on the surrounding gas and on the water content of the specimen;

"(*d*) The concentration of free radicals decreases relatively slowly after irradiation and is still well measurable for minutes or up to many hours according to the material and environmental conditions (water content and gas);

"(*e*) The opinion, widely held up to the present, that absorption of radiation in biological material generally leads within micro-seconds to states stable in the physical sense, must be abandoned;

"(*f*) It has been proved in some cases that a molecular interchange exists between protective substances and the protected material, and that it plays a fundamental part in protective action."

V. Chemical factors modifying radiation response in cells

Oxygen effect

94. The influence of oxygen tension on the response of biological systems to radiation is one of the fundamental phenomena of radio-biology. This influence, ex-

erted during irradiation, is generally called "oxygen effect". Gray's recent review integrates the data in this area.[182] The effect has been observed in a great variety of biological systems and can be described in the following way:

(*a*) In the absence of oxygen, or at reduced oxygen tension, the effects of radiation are diminished but not eliminated; oxygen acts as a dose multiplying agent. Considerable clarification of the quantitative relations between radio-sensitivity and oxygen tension has resulted from work with the bacterium *Shigella flexneri*.[183] Since, for this organism, survival is exponentially related to dose at all oxygen tensions, the slope of the curve may be used as a measure of radio-sensitivity. It has been found that, when a sufficiently dilute suspension of bacteria is vigorously bubbled throughout the period of irradiation with gases containing different percentages of oxygen, the relation between radio-sensitivity, S, and the concentration of oxygen (O_2) in the medium in which the organisms are suspended is fairly accurately represented by the simple relation:

$$\frac{S - S_N}{S_N} = (m - 1)\frac{[O_2]}{[O_2] + K}$$

where S_N is the sensitivity under anaerobic conditions, obtained by bubbling oxygen-free nitrogen through the solution, and m and K are constants. In general, m is the ratio between the effectiveness of a given dose when oxygen is freely available and the effectiveness when oxygen is absent. Thus, $(m - 1)$ may be considered as the ratio of the oxygen-dependent to the oxygen-independent components of radio-sensitivity. The constant K is the concentration of oxygen at which the sensitivity is exactly midway between anaerobic and fully aerobic values. The ratio m varies around 3 for a wide range of cell types and effects: inactivation of bacteria,[183–186] and yeast,[183] growth,[187] chromosome aberrations[189, 200] and mitotic delay[201] in plant tissues, as well as inactivation of isolated mammalian cells.[202, 203] The similarity between values of K (in the range of 4.5-5.0 μM/l) for irradiation of bacteria, yeast,[204] ascites tumour cells,[202] and plant root cells,[189] may be fortuitous, since a somewhat higher value of K (10 ± 2.8 μM/l) has been reported[205] for *Tradescantia* pollen tube chromosomes.

(*b*) In wet metabolizing systems, the presence of oxygen during irradiation appears to be essential since no effect has been seen in bacteria irradiated under anoxic conditions when oxygen is introduced only 20 milliseconds later.[206] Even stronger evidence is supplied by studies of the inactivation of *Serratia marcescens* by very short pulses of high intensity electron beams.[207] Cell suspensions were irradiated with 1.5 MeV electrons delivered either in a single pulse of two microseconds duration (10-20 krad total dose) or for five minutes at a dose-rate of 1000 rad/min; both treatments were applied either in hydrogen or in a 1 per cent oxygen and 99 per cent nitrogen mixture. When irradiation was very short, the radio-sensitivity of the bacteria was the same as under anoxic conditions, whereas with the longer irradiation, oxygen enhanced the sensitivity by a factor of 2.5. However, in dry bacterial spores two actions of oxygen, one realized only if oxygen is present during irradiation, the other at appreciable times after irradiation, have been shown.[52, 53, 118]

(*c*) Oxygen effect is usually less marked when cells are exposed to high LET radiation. An important aspect of the oxygen effect is that the enhancement ratio, m, varies with type of radiation, being highest with radiation of lowest LET.

95. The nature of radio-chemical reactions in the oxygen effect including the possible role of $HO_2^{\cdot}$ radicals and of other reactive products whose yields are influenced by oxygen tension, have been widely discussed in recent years.[208] Proof has been cited[52, 53, 118] that oxygen-free radical interaction takes place in bacterial spores to bring about biological damage by X-rays. However, the spores are semi-dry, and the role of water in these interactions has been studied as yet only in a preliminary fashion.[209, 210] Consequently, a generalization involving the metabolizing cell cannot be made now. The belief that the oxygen effect depends on cellular aerobic metabolism is challenged by experiments in microorganisms with normal and defective cytochrome systems in which oxygen effect is the same.[211] However, oxygen effect varies with the cell's physiological state. For instance, freshly harvested yeast cells, before starvation, have a considerably higher oxygen enhancement ratio ($m = 3.6$) than cells which have been starved. The ratio m decreases as the starvation period is prolonged, reaching a minimum value of $m = 2$ after two days' starvation.[212] The observation that oxygen alone causes chromosome aberrations when in high concentrations[213] complicates interpretation at this time.

96. This oxygen effect must not be confused with the effect of oxygen given in the post-irradiation period. Since the development of radiation injury depends on metabolism, it is likely that there are systems in which the magnitude of radiation lesions can be altered by changes in oxygen tension after irradiation.[214–217] Several papers have also dealt with the effect of anoxia; these have shown that anoxic conditions in metabolizing cells after irradiation reduce damage in some cases,[218] in others enhance it.[219]

Effect of gases other than oxygen

97. If oxygen exerts its radio-biological effects by reacting with radicals induced by radiation, other oxygen-like substances may react similarly.[125] In *Shignella flexneri* Y6R bacteria,[220] nitric oxide enhances radiation damage in the absence of oxygen. Nitric oxide has been found to enhance the effects of ionizing radiation on plant roots[221] and on ascites tumour cells.[222] In *Drosophila,* nitric oxide present during irradiation enhances the production of dominant lethals and sex-linked recessive lethals.[223] The system seems to differ from that in bacteria and ascites cells in that the same concentration of oxygen does not show an equivalent effect. Although these studies have shown that nitric oxide may frequently simulate oxygen, differences in the effects of the two gases have been shown in dry biological materials. Dry grass seeds irradiated and stored in nitric oxide are less affected by radiation than those irradiated in anoxia. However, when the water content of the seeds exceeds 12 per cent, nitric oxide is as effective as oxygen.[179] In spores of *Bacillus megaterium,* two actions of nitric oxide are known: a small sensitizing action during irradiation and a large protective action after irradiation.[121] The latter action is a consequence of removal of free radicals.[52, 124] The degree of hydration may influence the size of the two actions.[182]

Effects of gases under pressure

98. The oxygen effect on *Vicia faba* roots and ascites tumour cells is prevented when cells are irradiated in liquids in equilibrium with different gases under pressure.[224, 225] The following gases have this effect:

helium, hydrogen, nitrogen, argon, krypton, xenon, and cyclopropane; the same applies to nitrous oxide in tumour cells. The mode of action has not yet been established; the structures normally injured by radio-chemical reactions involving oxygen may be protected by an absorptive layer of the other gas. Proof that these substances interfere with injuries directly or indirectly dependent on oxygen is provided by the fact that they never reduce the effects of the oxygen outside the limits of anaerobic conditions. This research may provide a most valuable clue to the mechanism of oxygen effect.

Hydration

99. The precise significance of water radiolysis in the reactions induced in cells by radiation has still to be determined. New facts on this subject have been given by experiments of Hutchinson *et al.*[226] They measured inactivation of two enzymes (invertase, alcohol dehydrogenase) and of coenzyme A in wet and in dry yeast cells. They found that the sensitivity of these enzyme molecules were two times and twenty times greater respectively in the wet state, than in the dry state. Wet versus dry sensitivity for coenzyme A was estimated as 100 to 1. It has been assumed that the difference between the wet and the dry sensitivities is caused by the migration of chemically active intermediates formed by irradiation of water in the wet cells. Hutchinson[47] estimates that the migration distances of the water radicals are about the same (30 Angstroms) in all three cases.

100. Although increased water concentration enhances radio-sensitivity in *Aspergillus*,[227] several investigations[228–231] comparing radio-sensitivity of dried and wet plant seeds show that it is higher in the dried. Experimental results on *Artemia* eggs[232, 233] parallel results on plant seeds. It is difficult to draw a general conclusion from the few investigations made on the comparative radio-sensitivities of wet and dry cells. The possibility must be considered that, in some experimental conditions, radio-sensitivity is modified by an inadvertent change in oxygen tension within cells which is very likely to be different for different moisture contents. Also, it may well be that effects of moisture observed in plant seeds and *Artemia* eggs are due mainly to alterations in physiological state rather than to participation of water radicals in primary radio-chemical reactions.[231, 233]

Peroxide after-effects

101. If phage particles are irradiated in buffer and allowed to remain in the suspending medium after irradiation, the number of damaged particles increases with time.[234–236] Similar phenomena have been reported[237–239] in bacteria, in lysogenic systems, and in phage bacterium complexes. This after-effect may be attributed to the presence of H_2O_2 or of organic peroxides formed in the broth. However, doses exerting profound effects on whole cells are often not high enough to produce damaging concentrations of peroxides in the suspending media. This holds particularly if cells contain catalase, but hydrogen peroxide and organic peroxides in dilute suspensions which contain little protective organic matter may also exert a marked effect. In synthetic media, the concentration of peroxides responsible for the after-effect decreases with time during twenty days after irradiation. During this period the rate of decrease depends on dose, at least in the 1-5 kilorad range.[236] Artificially added inorganic peroxides, e.g. persulfate and urea peroxide,[240] can also increase sensitivity of phages and bacteria.

102. A possible clue to the action of peroxides has been found through studies of radiolysis of purines and pyrimidines. The addition of hydrogen peroxide and persulfate to irradiated solutions increases the G value of pyrimidines but leaves the G value of purines unaltered.[241, 242]

Chemical protection

103. Certain substances of different composition and distinct physical and chemical properties, when added to cell suspensions, can reduce the effects of subsequent irradiation. Study of the chemical protection of the cell is potentially helpful for understanding the primary events of radio-biological processes. Among "protective agents", the sulphur-containing compounds (cysteamine, cystamine, aminoethyl-isothiouronium, glutathione, etc.) are the more important. A few inhibitors of enzyme activity (sodium cyanide, sodium azide, etc.), some metabolites (gluconate, pyruvate, ATP)[243–246] and alcohols,[247–249] have the same action. Chemical protection requires the presence of the protector before or during irradiation, and is more effective against X-rays than against other ionizing radiations. However, some metabolites can also have positive effects after irradiation, possibly by influencing repair processes.[245, 246]

104. Protection has for long been associated with the indirect action of radiation. It has even been used as a criterion for distinguishing indirect from direct action. This view can no longer be justified. Experimental evidence has been presented wherein no indirect action can be envisaged.[141, 250–252]

105. One action of protective agents may be explained by a decrease of oxygen tension.[253, 255, 256] The anoxic hypothesis implies utilization of oxygen by the protector, e.g. in transformation of cysteamine into cystamine. Support for an anoxic effect of protective agents stems from experiments in which the dose reduction factor with cysteamine is similar to that of simple oxygen removal.[254] However, several investigators consider that sulphydryl compounds are protective by other means than production of anoxia. The most recent observations supporting this have been obtained in *Escherichia coli*,[256–258] in isolated rat thymocytes,[259] and in HeLa cells in tissue culture.[260]

106. Alternatively, protection may be achieved by combination of the chemical protector with free radicals produced by irradiation. By comparison with chemical data[132] a competitive type of reaction may be envisaged. This reaction involves free radicals, oxygen, and protector. The protecting molecule may act either by combining with free radicals, thus avoiding formation of an unstable active peroxy-radical, or by attacking the peroxy-radical and making it stable, i.e., non-active.[261] No clear-cut evidence has been presented in favour of either hypothesis.

107. Another explanation is that protecting molecules attach themselves primarily to cell structures, thus masking sensitive sites. The complex so formed would guard these sites from the attack of free radicals (indirect action). This complex may also dissipate absorbed energy less harmfully (direct action). With SH-containing compounds, Eldjarn and Pihl[243] have proposed a chemical model embodying this concept. The masking-effect hypothesis is supported by experimental results showing that decrease of protective ability of cysteine injected into animals parallels recovery of the metabolic activity which that substance had initially lowered.[262, 263]

108. Other substances with known pharmacological activities (hormones, amines, neurodrugs), protectors after injection in animals, seem to have no action in cell suspensions. Thus, little information about the primary events of radio-biological action can be obtained from *in vivo* experiments in which they are used except for that concerning their possible interference with metabolic processes.

109. The chemical protective agents are also effective against chromosome aberrations[264] and induction of mutations by X- and gamma rays.[265,266] However, this subject deserves much more attention, the data being scanty.

110. Accumulated evidence on chemical protection[243,244] does not now permit an unequivocal recognition of mechanism. New data are needed to clarify this. The ESR technique may become useful in this area.

VI. Effect of radiation on cellular structures and their function

111. Some of the more spectacular and most extensively studied effects, such as inhibition of cell division, mitotic delay and mutation, are most readily associated with nuclear damage and are apparent after exposure to relatively small doses of radiation. However, inhibition of cytoplasmic functions should be carefully considered in assessment of total damage. Since nuclear and cytoplasmic functions are so clearly intertwined, it is imperative to consider their possible interactions in weighing the relative importance of nuclear and cytoplasmic damage.

112. These interrelationships vary with different systems and different functions. The early works of Winternberger,[267] Zirkle,[268] Henshaw,[269] Hercik[270] and Petrova[271] showed that mitotic delay and cell death are principally manifestations of radiation damage sustained by the nucleus. Recent experiments dealing with partial cell irradiation have shown clearly that irradiation of genetic material is far more effective than cytoplasmic irradiation in producing cell lethality. For example, 50 per cent inhibition of hatching of *Habrobracon* eggs requires 10^7 alpha particles to the cytoplasm; only 1 alpha particle to the nucleus suffices to inactivate the egg.[272] Comparable results have been obtained in similar experiments with newt heart cultures.[273] Conversely, situations may be expected where cytoplasmic damage is relatively more effective in impairment of specific cell functions. For example, changes in isoelectric point of mitochondrial nucleoproteins of the adult nerve cell occur during or immediately after irradiation with small doses.[275–277] This indicates alteration of metabolic functions and, in particular, of oxydative phosphorylation.[275,278]

113. Non-nucleated cells (*Acetabularia*, amoebae,[279] *Paramecia*,[280,281] tissue culture cells)[282] ultimately die, but they may survive for a considerable time and even continue to differentiate (*Acetabularia*).[280,283,284] Lethally irradiated *E. coli* cells retain the ability to synthesize active bacteriophage.[236,285–288] Owing to this high degree of cytoplasmic autonomy, nuclear radiation damage affecting cytoplasmic functions may escape detection during the observation period.

114. Conversely, cytoplasmic damage affecting the physiology of the cell may not become permanent if the "genetic" or "non-genetic" factors necessary for recovery of the damaged structure are functional. The contribution of the cytoplasm in radiation injury has been partially clarified by recent investigations. In particular, the presence of toxic products[289–290] and the existence of changes in IEP (isoelectric point) perhaps associated with changes in RNP (ribonucleoproteins) localized in cytoplasmic microstructures may imply disturbances in nuclear cytoplasmic interaction.[291–296]

115. Particular emphasis has been placed on the metabolism of deoxyribonucleic acid (DNA) and on its interaction with ribonucleic acid (RNA) and protein metabolism. These metabolic functions are so intimately intertwined in the way they influence cell division and replication that it seems logical to treat them integrally to assess how radiation may affect this complex.

DNA SYNTHESIS

116. Recently Kornberg and associates[297–299] have synthesized DNA *in vitro* from deoxyribonucleoside triphosphates using purified extracts from *E. coli*. The system requires "primer" DNA which, during the reaction, replicates. The product has a base composition identical with that of the native primer. Single stranded (denatured) DNA preparations also provide excellent primers.[300]

117. This mechanism is compatible with present concepts on DNA replication *in vivo*. These postulate that double-stranded DNA may split wholly or partially into single strands that serve as templates and receptors for complementary strands. Moreover, Kornberg *et al.*,[301] identifying all the dinucleotides in synthetic DNA, have shown that the *in vitro* system produces double-stranded DNA molecules with each single spiral running in the opposite direction as compared with its mate; this result provides excellent support for the Watson Crick model.

118. The presence of polymerase, first found in *E. coli* extracts, has also been demonstrated in extracts of mammalian cells from ascites tumours, thymus, regenerating liver, etc.[302–305]

119. In the nuclei of tissue cells, DNA synthesis is limited to a definite period during interphase. In the first hours after mitosis there is usually no DNA synthesis (G_1-period). In the next period (S-period), lasting several hours, the DNA content of the cell doubles. The interphase is concluded by the G_2-period. This sequence of events in the interphase may be subject to modifications; thus, in ascites tumour cells the G_1-phase is absent. Precursors of DNA are probably produced in the G_1-phase and activated (to nucleosidetriphosphates) at the expense of energy-generating processes (e.g. nuclear oxydative phosphorylation). Nuclear synthesis of RNA also occurs in this phase, associated with the production of new enzymic proteins. In the synthetic period, the assembly of activated precursors most probably occurs with the help of the newly synthesized enzymes and with the original DNA serving as template and primer. In the G_2-period DNA is further prepared for its subsequent role in the imminent cell division. In cells of lower organisms this stratification into well separated division stages does not occur. Probably, however, the sequence of metabolic events is similar.

120. Since the discovery by Hahn and Hevesy[306] that phosphorus incorporation into DNA is inhibited by ionizing radiation, a fact confirmed by similar evidence on incorporation of various labelled precursors such as

adenine, orotic acid, formate, phosphate and thymidine, it has been generally accepted that DNA synthesis is a particularly radio-sensitive metabolic process. Recent investigations have cast serious doubt on the correctness of this opinion. They lead, rather, to the conclusion that relatively low radiation doses do not affect the rate of DNA synthesis in various types of cells. It is now realized that a diminished incorporation of precursors into DNA after irradiation may not necessarily represent primary inhibition of DNA synthesis. It may be the consequence of other differences between the irradiated and the control cell populations,[307-311] namely:

(*a*) Accumulation of cells in the G_2-phase as a result of mitotic inhibition;

(*b*) Changes in the distribution of the various cell types of a mixed cell population;

(*c*) Increase of the fraction of dead cells in the irradiated population. The same argument obviously applies to the synthesis of RNA and protein.

121. Recent developments in the use of microspectrophotometry and autoradiography for the study of single cells often make it possible to account for these complications and thus to arrive at a more correct evaluation of the biochemical effects of irradiation. Another method, although at present often more difficult, uses more or less synchronously dividing cells. The following survey considers investigations using these techniques.

122. Irradiation of HeLa-cells with 550 r leads to a considerable increase in the fraction of cells synthesizing DNA as compared with control cultures.[312] This increase amounts to 100 per cent six hours after irradiation (this represents a larger fraction than can be accounted for by inhibition of mitosis). Apparently, cells irradiated during active DNA synthesis continue to synthesize for longer periods than normal; this may be related to giant cell formation. Moreover, Painter[313] found that when post-irradiation mitosis resumes, added tritiated thymidine results in a lower fraction of labelled cells in mitosis of these cells than in mitosis of unirradiated controls. This could be due to sluggishness of irradiated cells in the G_2-phase and/or in mitosis of the next division stage.

123. In contrast, Harrington[314] did not see any direct effect of exposure to 500 r on the fraction of U-12 fibroblasts in DNA synthesis. The percentage of cells synthesizing DNA began to drop after an interval corresponding to the duration of the G_1-phase; this decline must be wholly attributed to inhibition of mitosis.

124. A similar conclusion has been drawn from studies of L cells (mouse fibroblasts)[315,316] in which DNA synthesis continued in the absence of mitosis until the double premitotic content per cell was reached. Very high doses (4000-5000 r) retarded DNA synthesis instantaneously. After exposure to 2000 r the cells still completed an average of three divisions, whereas after 5000 r, only 20 per cent of the cells were still capable of a final division. Such DNA synthesis as was observed thereafter was in giant cells and occurred at a considerably lower rate than in normal unirradiated cells.

125. X-irradiation (800-1250 r) of Ehrlich ascites tumours has not been found to inhibit DNA synthesis.[317,318] Mitotic activity is arrested instantaneously but volume, dry weight and total nucleic acid per cell continue to rise considerably. The DNA content per cell rises to the pre-mitotic level. Harbers and Heidelberger[319] cultured and irradiated Ehrlich ascites tumour cells *in vitro* using doses of 750-3000 r. They found inhibition of the incorporation of (2-C^{14}) uracil in DNA thymine, but the possibility that this effect was due to inhibition of mitosis has not been excluded. Further results have been reported by Budilova[320] on the incorporation of several precursors into DNA molecules of isolated thymus cells nuclei; incorporation was greatly reduced in nuclei irradiated *in vivo*, whereas there were no changes when nuclei were irradiated *in vitro*.

126. In bone marrow cells *in vitro*, high doses of radiation ($>$ 500 rad) directly inhibit DNA synthesis. Lower doses ($<$ 300 rad) cannot inhibit DNA synthesis in cells already in the synthetic period. However, cells in the G_1-phase at the time of irradiation enter the S-phase only after an appreciable delay. More recent observations by Uyeki[321] are in accord; the number of cells entering DNA synthesis after 800 r is strongly depressed.

127. Low doses of X-radiation (50-140 r) prevent division of root tip meristem cells of *Vicia faba* but do not interfere directly with DNA synthesis.[322,323] However, cells not yet in synthesis at the time of irradiation pass on to the synthetic phase only after a delay of 10 hours or more. In contrast, Das and Alfert[324] have reported an immediate effect of irradiation on DNA synthesis; even a dose as low as 200 r enhances DNA synthesis, whereas 800 r increases the uptake of tritiated thymidine to approximately five times the control value.

128. From studies in regenerating liver[325,326] it has been concluded that DNA synthesis itself is not primarily affected after partial hepatectomy by relatively feeble radiation doses.[325,326] In resting liver there is no appreciable DNA synthesis, but when regeneration is induced by partial hepatectomy, synthesis begins 15-18 hours after the operation and reaches a maximum at 24-29 hours. In this first stage of regeneration there is reasonable synchronization of DNA synthesis. High radiation doses (up to 2,000-3,000 r) are needed to inhibit synthesis once it has begun; a dose of 500 r is ineffective. However, the latter dose is quite effective in postponing synthesis when given before the beginning of the synthetic period.

129. Few experimental data are available on the sensitivity of DNA synthesis in micro-organisms to X-irradiation. Billen[327] studied mutants of *E. coli* and, in particular, the influence of "unbalanced growth" and radio-sensitivity. He concluded that X-irradiation inhibits the synthesis of protein required for DNA replication.

130. In dividing *H. influenzae*, *E coli* B and B/r, irradiation with doses between 19 and 100 k rad is followed by breakdown of cellular DNA; after a certain time this process stops and is followed by an increase in DNA.[328,329]

131. In *H. influenzae*, the biological activity of DNA, as characterized by its transforming activity, has been determined after irradiation. All remaining DNA and DNA formed after irradiation is functionally normal. No relation has been found between killing and severity of DNA breakdown. From this it has been concluded that observed DNA breakdown is not the immediate radiation-induced process leading directly to cell death.[329]

132. DNA is in a highly polymerized state in bacteriophages[330,331] and certain tissues.[332,333] After irradiation, depolymerization is seen,[333-335] and shifts in the purine/pyrimidine ratio in DNA synthesized after X-irradiation of spleen cells *in vivo* have been observed.[336,337] Changes

in the thymine/adenine ratio in DNA synthesized after irradiation of plants have been reported by Kusin and Tokarskaya.[338,339] These changes seem to be closely related to disturbances in nucleotide metabolism.[340-342]

RNA AND PROTEIN SYNTHESIS

133. In contrast to DNA, most RNA is in the cytoplasm; only a small fraction resides in the nucleus.

134. Little is known about the secondary structure of RNA. It is probably single-stranded. Physico-chemical data suggest that it may fold locally into incomplete double spirals stabilized by H-bonds; these orderly structures would be held apart by unarrayed segments of the RNA chain.[343]

135. Nuclear RNA is not homogenous; an important fraction is probably in ribosomes, as observed in thymus nuclei. Cytologically, RNA may be divided into chromosomal and nucleolar RNA. Biochemically, two fractions of nuclear RNA may be distinguished, one extractable by low concentrations of saline (n-RNA_1), another remaining undissolved (n-RNA_2). Generally, n-RNA_1 incorporates labelled precursors less readily than does n-RNA_2.[344-346] According to Zbarskii and Georgiev[347,348] n-RNA_1 represents the chromosomal RNA and n-RNA_2 forms part of nucleolar RNA.

136. In the cytoplasm, RNA occurs in the cell sap (S-RNA) and in the microsome (liver, pancreas) and ribosome fractions. The molecular weight of S-RNA is relatively small (20,000-40,000); that of microsomal RNA is considerably larger (approximately 1.7×10^6). The possibility cannot be excluded that the latter molecular weight represents aggregates of molecules of lower molecular weight as it has been shown that ribosomes may disintegrate into smaller particles depending on the Mg^{++} concentration of the solvent. The RNA in the smallest ribosomes, the so-called 30 S particles, has a molecular weight of only 5.6×10^5. Small amounts of rapidly turning over "messenger" RNA of an intermediate size, between the latter RNA and S-RNA, are present in uninfected and phage-infected bacteria.[349,350] This RNA attaches itself to existing ribosomes and confers on them the code for protein synthesis.

137. Recent studies provide evidence that RNA is synthesized exclusively in the cell nucleus, and is transported from nucleus to cytoplasm after synthesis. Thus, Goldstein and Plaut[351] transplanted P^{32} RNA labelled nuclei from intact amoebae into enucleated amoebae; after a while the cytoplasm of the host contained labelled RNA. As these amoebae were viable, it seems unlikely that leakage from damaged nuclei was responsible for the effect.

138. So far, the type of the nuclear RNA transported into the cytoplasm has not been established. Woods and Taylor[352] have suggested that RNA is primarily synthesized in chromosomes and subsequently stored in the nucleolus; from there it would be transferred to cytoplasm. This hypothesis is supported by other investigators[353,354] who have found that, with a labelled RNA precursor, radio-activity is first detected in chromatin and only later in the nucleolus; continued incubation in the absence of labelled precursor leads to an earlier and faster fading away of the radio-activity of the chromosomal than of the nucleolar RNA.

139. Whether this hypothesis has general validity for all types of cells is not known. From experiments on selective irradiation of the nucleolus by UV microbeams, Perry *et al.*[355] have concluded that RNA transport into the cytoplasm originates from both nuclear locations of RNA. From recent autoradiographic studies of the incorporation of tritiated precursors into RNA of HeLa-cells, in which several correction factors were applied for the conversion of grain counts into actual incorporation, the same authors state that their data do not show a transport of RNA from chromatin to nucleolus.[356] Moreover, a few instances are known where labelling of the nucleolus precedes that of the chromatin.[357]

140. Little is known about the mechanism of RNA synthesis. An enzyme, polynucleotide phosphorylase, that catalyzes the synthesis of RNA from ribonucleoside diphosphates has been found in micro-organisms by Ochoa and associates.[358] The purified enzyme requires a primer, but any tri- or tetranucleotide may serve in this capacity, and it is not the primer but the available nucleotide diphosphates that determine the base composition of the product.[359-361]

141. On the other hand, extracts, not only from micro-organisms but also from animal cells, polymerize ribonucleoside triphosphates to RNA.[362,363] When DNA is present, treatment with DNA-ase destroys its activity. Enzymatic activity depends also on the simultaneous presence of the triphosphates of all four nucleosides. Furth *et al.*[364] and Weiss and Nakamoto[365] have shown that newly synthesized RNA is a copy of the base composition of the added "primer" DNA. The enzyme produces polyadenylic acid or poly-uridylic-adenylic acid when primed with polythymidylic- or poly-adenylic-thymidylic acid respectively. With *M. lysodeikticus* or T_2-DNA as a primer, the newly synthesized RNA has the same nearest-neighbour base frequency as the primer.[366] The resemblence of this enzyme to the polymerase of DNA synthesis is striking.

142. From experiments with labelled RNA precursors, it has been shown that synthesis of RNA occurs during the entire interphase, although in some cells the process is slower during S-phase. During mitosis, no RNA seems to be synthesized.

143. Within the nucleus, DNA transfers its genetic information to RNA.[367,368] The presence of an RNA polymerase requiring DNA for action, and copying its base composition, supports this concept. RNA formed in the nucleus then passes into the cytoplasm, carrying its information to protein synthesizing sites. Rich[369] has demonstrated that, in principle, a single-stranded RNA molecule can unite with a complementary single-stranded DNA molecule. Moreover, Hall and Spiegelman[370] have shown specific hybrid formation between single-stranded T_2-DNA and the RNA synthesized subsequent to infection of *E. coli*. Geiduschek *et al.* do not favour single-stranded DNA as a necessary intermediate in RNA synthesis *in vitro*.[371]

144. Apparently, the base sequence of the DNA is transcribed into newly formed messenger RNA, triplets (or multiples of 3) of nucleotides carrying the information for various amino acids (para. 151). The most direct proof of the ability of RNA to carry genetic information is provided by the information that purified tobacco mosaic virus RNA is apparently infectious. How information transfer between DNA and RNA is effected is not known. Leslie[372] recently postulated, from studies on human liver cells and from the literature, that coding for micro-organisms and for somatic cells of higher organisms may differ.

145. About twenty years ago, a relationship between RNA and protein synthesis was independently advanced by Caspersson[367] and Brachet[368] as a hypothesis; this hypothesis has now become a firmly established biological concept.

146. Protein synthesis has been most studied in microorganisms and in the microsomal fraction of the cytoplasm of higher cells. The first step is activation of amino acids in a reaction with ATP resulting in an amino acid adenylate. The latter compound does not appear freely in solution but remains attached to the enzyme; amino acid activation is therefore usually studied from the exchange between labelled pyrophosphate (one of the reaction products) and the phosphate groups of ATP or by the chemical transformation of the amino acid adenylate by hydroxylamine into hydroxyamic acid.

147. The activated amino acid then becomes attached to the transfer or soluble RNA (S-RNA). It is bound in the manner common to all amino acids, via the terminal nucleotide sequence cytidylic-cytidylic-adenosine; the amino acid residue is bound in ester linkage to the C_3'-atom of adenosine. Although the method of binding is identical, each amino acid has a high specificity for the S-RNA to which it becomes attached. There are different S-RNA molecules for each type of amino acid. The specificity of S-RNA resides in its base sequence.

148. The function of S-RNA is that of acting as a carrier which brings the amino acid to the template. Investigations of Bosch *et al.*[373] have shown that S-RNA can be firmly bound to the ribosomes. On the other hand, it is possible that this "transfer"-RNA resides permanently in the ribosomes. Thermodynamically, this latter hypothesis is more attractive; it may be significant that in one of the very scanty examples of net synthesis of enzymatically active protein *in vitro* this could be accomplished by a cell-free system in which S-RNA formed part of the ribosome particles.[374]

149. The last phase in protein synthesis is the assembly of activated amino acids into polypeptide chains by peptide linkages, and release of these chains from ribosomal particles. For this step GTP is required. The process is greatly stimulated by SH-compounds.[374, 375]

150. Protein synthesis has been studied in microsomes of cells of higher organisms. It is, however, by no means confined to this system. Net synthesis of cytochrome-c has been demonstrated by Bates *et al.*[376] in mitochondria. Moreover, it has been shown by Allfrey and Mirsky[377] that protein synthesis in the nucleus is very similar to that in the cytoplasm. These investigators suggest that the energy for protein synthesis in the nucleus is provided by phosphorylation in mitochondria.

151. The part played by RNA in carrying genetic information for the production of proteins is clearly shown by the discovery of Astrachan and Volkin[378] that infection of *E. coli* by various bacteriophages immediately induces the production of a new RNA which resembles, in base composition, the DNA of the phage. Nomura *et al.*[379] found that, after T_2 infection, there is no synthesis of typical ribosomal RNA and that phage specific RNA sediments at a slower rate (8 S) than ribosomal (16 S and 23 S). Apparently, the genetic information for the synthesis of phage protein does not reside in the usual ribosomal RNA but is induced in pre-existing ribosomes by a phage specific RNA which may be considered a messenger RNA. Brenner *et al.*,[350] using isotope labelling techniques followed by careful separation of the various RNA-containing fractions, actually demonstrated that the new RNA (which, according to Volkin and Astrachan,[378] has a base composition corresponding to that of the phage DNA) is associated with pre-existing ribosomes and provides them with the necessary information for specific protein synthesis. Gros *et al.*,[349] in "pulse experiments" with tracers, have shown that exactly the same situation prevails in uninfected bacteria where an RNA component with rapid turnover and which is physically distinct from ribosomal RNA or S-RNA can be demonstrated. The fraction behaves in the ultracentrifuge and towards pre-existing ribosomes in high Mg^{++} concentrations exactly as the phage specific RNA induced by T_2 infection; it becomes associated with the active 70 S ribosomes, the site of protein synthesis. According to this concept, the typical ribosomal RNA carries no genetic information. The concept of messenger-RNA has been greatly elucidated and amplified by experiments of Matthaei and Nirenberg[380] who demonstrated that, in cell-free extracts of *E. coli* containing ribosomes, poly-urydylic acid can induce the synthesis of poly-phenylalanine. At present, triplet code letters have been assigned by Speyer *et al.* to 14 amino acids.[381]

152. The influence of ionizing radiation on RNA and protein synthesis has not been studied to the same extent as that on DNA synthesis, and available data do not permit a satisfactory analysis of the effects.

153. Painter,[313] using 1,500 r, did not find a significant disturbance of the uptake of tritiated cytidine into the RNA of HeLa cells. Neither did Harrington[314] see any effect on the incorporation of tritiated cytidine into nuclear RNA of U 12 fibroblasts after 500 r. Shabadash, on the other hand, showed that cellular ribonucleoproteins are extremely responsive to penetrating radiations.[277, 291] This was recently confirmed biochemically.[295] Ribonucleoproteins localized in structures of different organelles do not have identical physico-chemical properties, as indicated by differences in their iso-electric points,[382] which are more acid in mitochondria than in microsomes. The former is more sensitive to penetrating radiation.[293, 296]

154. Klein and Forssberg[321] irradiated Ehrlich ascites tumour cells *in vivo* with 1,250 r and found no changes in RNA synthesis. However, *in vitro* irradiation of these cells inhibits incorporation of labelled uracil into RNA of the nucleus but not into that of the cytoplasm.[319] This result is difficult to understand in view of the probable nuclear origin of most RNA.

155. From the studies of Logan and collaborators,[383, 384] it has been concluded that irradiation of isolated liver and calf thymus nuclei *in vitro* distinctly reduces the rate of incorporation of labelled precursors into nuclear RNA. A similar effect on the incorporation of P^{32} into nuclear RNA can be obtained with regenerating liver, if irradiation is given at the earliest stage of regeneration.[385] This observation agrees with data on the synthesis of certain enzymes necessary for the synthesis of DNA in regenerating liver. Thus, Bollum *et al.*[386] have found the synthesis of the enzymes DNA polymerase and thymidine kinase to be inhibited by radiation doses of 375-1,500 r if irradiation is given 6 hours after partial hepatectomy. The same doses, given sixteen hours after the operation, are ineffective. Other authors have also found that polymerase synthesis is inhibited by irradiation in the first phase of the regeneration process.[387, 388]

156. Relatively low doses of radiation can postpone the onset of DNA synthesis in various types of cells.

It seems reasonable to assume that inhibition of enzyme synthesis is at least one cause of this delay.

157. Ionizing radiation also reduces the synthesis of enzymes in micro-organisms. Pauly[389] has reported a 37 per cent dose of 7×10^4 r for the inhibition of the induction of lysine decarboxylase in *Bacterium cadaveris*. Radio-sensitivity was the same for the rate of synthesis and the maximum level of enzyme formed. This finding leads to the conclusion that every cell possesses one or more "centres of synthesis", each producing a definite number of enzyme molecules. These synthetic centres would be destroyed according to single-hit kinetics. The induction of catalase by O_2 in a diploid mutant of *S. cerevisiae*, however, is stimulated by a radiation dose of 10^5 r. This stimulation may be due to the production of peroxides in the cell, as suggested by Chantrenne and Devreux.[390] Using serological techniques and also various tagged amino-acids in newly synthetized proteins of individual organelles of cells, Ilina and Petrov[391, 392] showed that qualitatively altered proteins are formed after irradiation.

Effects of radiation on antibody synthesis

158. Inhibition of antibody formation is a special case in the formation of specific proteins, and appears to be highly radio-sensitive. It involves the formation of a specific protein complementary in structure to the inductor antigen. The normal processes of antibody formation are only just beginning to be understood, and a generalized theory has still to emerge from several contradictory hypotheses. Antibodies are formed in the plasma cells of lymphoid tissues which themselves originate from undifferentiated cells of the reticular system. The mechanism of radiation inhibition of antibody formation, recently reviewed,[393, 394] thus must account for:

(*a*) The effect of radiation on the multiplication and differentiation of these reticular cells and their descendants;

(*b*) The process of antibody synthesis, which probably occurs in the microsomes of plasma cells.

159. One of the characteristics of radiation is its greater efficiency in inhibiting antibody production when administered prior to the antigen. The final titer of antibody is lowered only if irradiation occurs some hours before antigen injection. In this case, and also when irradiation takes place immediately before or after antigen injection, the latent period before the titer begins to rise is increased and the rate of synthesis decreased. Taliaferro[395] has distinguished a highly radio-sensitive (effects become detectable on the final titer for doses of 100 r) pre-induction period but this is not well defined in cytological or biochemical terms. The cause of this inhibition could be twofold:

(*a*) Decreased production of plasma cells from their "reticular ancestors", or from other types of cells also involved in the process;

(*b*) Delay and inhibition of the synthesis of new protein when antigen is injected.

160. Stevens[396] has shown a correlation between depression of the number of plasma cells formed after irradiation and inhibition of antibody synthesis. Furthermore, experiments by Taliaferro suggest that antibody formation depends on cell multiplication in irradiated animals; this does not exclude the possibility that *specific* effects on the induction of synthesis of new proteins are also involved. The antibody-producing period appears to be more resistant to radiation. Apparently, antibodies formed when the system is irradiated during this period do not differ fundamentally from normal antibodies. Studies of the degree of radiation sensitivity of the secondary response to antigen injection have yielded conflicting results; there have been several explanations, each of which might be acceptable for the particular antigen studied.[393, 394]

General considerations of radiation effects in cellular metabolism

161. The importance of radiation effects that are closely linked with cell division and replication, and which include mitotic inhibition, loss of reproductive power and mutations, has been stressed. It would be attractive to describe these changes within the frame of a unitarian mechanism, although such a treatment would be arbitrary. At least two key effects indicate a disturbance in the genetic properties of the cell.[397] One of these is the production of mutations. The other is that delayed effect on cell division in which cells multiply immediately after irradiation but nevertheless fail to form macroscopic colonies.

162. The failure of cells to divide even once when given higher radiation doses is also probably due to damage of genetic material. The inhibition of mitosis might be explained similarly, although here the implication that genetic material may be directly involved is less obvious. Much may be said for the concept that the main radiation effects are at some stage mediated through DNA; this explains why emphasis is laid upon the metabolism of DNA. DNA synthesis has been used in a restricted sense throughout this report to indicate the stage where precursors are assembled into polynucleotides. Subsequent stages may include many more biochemical reactions before the full-fledged DNA-protein molecule is formed and incorporated into daughter chromosomes. These late stages of DNA metabolism presumably take place in late interphase and in prophase.

163. There is some evidence, at least with radiation-induced mitotic delay, that the G_2 stage and early prophase may be the most radio-sensitive stages in the mitotic cycle of many cells.[398] Painter's work,[313] mentioned earlier, may also be interpreted in this way. The dependence of radio-sensitivity on division stage may not always prevail in somatic cells of higher organisms;[399] survival curves of somatic mammalian cells usually show no evidence of resistant fractions.[400] Because of considerable radio-sensitivity during the G_2 period, metabolic processes during this period are important. Unfortunately, biochemical knowledge of G_2 and subsequent mitotic stages is still extremely scanty. Therefore it is not yet possible to describe the effect of radiation at a molecular level on these phases.

164. In cells of higher oragnisms two patterns of synthesis of DNA probably occur. In tissue cultures and ascites tumour cells, DNA synthesis continues more or less unhampered if irradiation occurs during *any* period of the division cycle, at least when doses are not excessive; in cells of bone marrow, plant root tips and regenerating liver, DNA synthesis may be delayed when lower doses of radiation are delivered before synthesis has begun. This latter effect is probably due to inhibition of the formation of necessary enzymes as a result of interference with RNA synthesis. No inhibition, and sometimes even acceleration occurs in either pattern when all ingredients are available for synthesis. Mitotic inhibition interferes eventually because a feed-back

homeostatic mechanism precludes, or at least inhibits, DNA synthesis beyond the premitotic level.

165. This concept has been confirmed by Lajtha *et al.*[310] and by Berry *et al*;[401] they found that dose-effect curves for inhibition of DNA synthesis in bone marrow cells differ from those in ascites tumour cells. For bone marrow cells the curve has two exponentials, a "sensitive" one and an "unsensitive" one, characterized by 37 per cent doses of 500 and 1,300 r respectively. The curve for ascites cells lacks the sensitive component. Ord and Stocken[402] have, from similar curves for thymus tissue, suggested that the sensitive component may represent the inhibition of nuclear phosphorylation described by Creasey and Stocken.[403] This inhibition would lead to a shortage of DNA precursors. However, there is no evidence for such a shortage; Ord and Stocken[404] reported an accumulation of deoxyriboside mono- and triphosphates after irradiation of the thymus. The significance and reproducibility of the inhibition of nuclear phosphorylation seems doubtful.

166. Both cell types also differ in ploidy; tissue-culture and ascites tumour cells are usually aneuploid. The problem of the relationship between ploidy and radio-sensitivity is complex (para. 182) but it is not impossible that the high resistance of these cells may be a consequence of the aneuploidy. This suggests that DNA itself is the primary target. The work of Opara-Kubinska *et al.*[405] and many studies on bacteriophages indicate that this is probably so, at least for transforming activity and survival in micro-organisms.

167. The "primer" function of DNA in RNA synthesis by the RNA polymerase enzyme means that the explanation given for the delay of DNA synthesis, namely interference with RNA metabolism, is at least not incompatible with a primary radiation lesion in DNA itself (in this case, the primer) (para. 155). This does not exclude the possibility that effects on DNA-RNA protein metabolism, even when mediated through DNA, may not result secondarily from quite another primary radiation lesion, e.g. lesions on larger subcellular structures, proteins, membranes, lipoids.

Effects of radiation on integrated functions

168. When irradiated in comparable conditions, different cellular populations react in similar patterns. With increasing doses, effects often become experimentally measurable in the following order: modifications of growth rate, mitotic delay, inhibition of mitosis, delayed or reproductive death and interphase death.

Growth rate

169. Under chronic irradiation, the total mass of cell cultures first increases and then decreases.[406–408] The initial increase of the total cell mass of the culture accompanies the emergence of giant cells, the volume and usually the ploidy of which increase without division. This phenomenon has been observed among bacteria, yeasts and mammalian cells, and seems therefore to be fairly general. As dose accumulates, the total weight of the culture diminishes and becomes lower than that of controls. In general, radiation reduces growth rate and increases generation time; however, under certain metabolic conditions, the generation time can be shorter than in control cultures once irradiation is discontinued.[409,410] Interference with growth rate has also been detected in isolated cells. In *Phycomyces blakesleeanus*, Forssberg[411] has shown a lowering of the growth rate of sporangiophores with extremely low doses of ~ 0.001 r.

Mitotic delay

170. When a cell has been irradiated before prophase, division is delayed. This delay can be modified by dose rate[412] and by oxygen concentration; this may mean that metabolic processes are involved.[413] The most informative experiments have been those of Carlson and Gaulden[414] with neuroblasts of grasshoppers' embryos. During mitosis there is a critical stage coinciding with the condensation of chromosomes into visible filaments and with the disappearance of the nuclear membrane and nucleolus. If a dose as low as 1 r is given to a cell before that critical stage, development of mitosis is delayed. However, this delay does not occur when the same or an even slightly higher dose is given later. In this latter case subsequent mitoses are delayed. More recent experiments have shown that the critical stage may be somewhat earlier in the mitotic cycle, i.e., in mid-prophase. Gaulden irradiated one of the two nucleoli of neuroblasts with a UV-microbeam and concluded that all cells treated at stages from late telophase to the middle of mid-prophase immediately show a permanent cessation of mitotic progress. This picture of mitotic delay looks slightly different when other types of cells are studied. In particular, the critical sensitive period and the duration of the various phases of mitosis may differ in different types of cells. In consequence, precise comparisons are difficult.

171. The main characteristic of mitotic delay is its temporary nature. Although the mechanism of mitotic delay is still far from being understood, some attempts have been made to explain it. Since DNA metabolism is known to be affected by radiation, it is tempting to attribute mitotic delay to inhibition of DNA synthesis.[415] This explanation is speculative, and it may well be that reduction in DNA synthesis, when observed, is the consequence rather than the cause of mitotic delay. In particular, the radio-sensitive period for producing mitotic delay usually occurs when DNA synthesis is already complete. In some instances, DNA metabolism is apparently normal despite inhibition of cellular division, e.g. in irradiated mammalian cells in tissue culture. This suggests that delay in division may be a consequence of injury to an unknown mechanism controlling the onset of division,[416] and that there is no direct involvement of DNA synthesis. Yamada and Puck showed that a reversible mitotic lag is produced by a block in the G_2 period after X-ray doses of 34-135 r in hyperploid S 3 HeLa cells.[274] They proposed that this reversible mitotic lag, like irreversible reproductive death, is due to chromosomal damage, and that the reversible lag may reflect interference with chromosomal condensation just before, and perhaps in, the early stages of mitosis. Other hypotheses have also been advanced: interference of radiation with oxydo-reduction of sulphydryl compounds produced during cellular division,[417,418] and inhibition of the division mechanism of the cytoplasm[419] or of the formation of the spindle.[420] Production of anti-metabolites may be responsible, as suggested by Kuzin,[296,421–424] who used plant material from which he was able to demonstrate antimitotic quinones.

Inhibition of mitosis and cellular death: reproductive and interphase death*

172. With increased doses, cellular death usually occurs. Cells can be killed either immediately (interphase

* Under doses higher than 100,000 rad, instantaneous death is observed, due mainly to protein coagulation.

death) or after a few divisions (delayed or reproductive death). In general, the doses required to achieve interphase death are higher, although there are cells which undergo interphase death even if irradiated by relatively small doses, e.g. small lymphocytes, primary oöcytes in insects and mammals, mammalian neuroblasts, insect ganglia cells. Reproductive death occurs in bone-marrow, intestinal crypt cells, lymphomas and spermatogonia.[425] It should be noted that the latter group consists of cells with a high mitotic index; with these, interphase death would probably require a higher dose.

173. The processes leading to reproductive or to interphase death are still unknown; it is likely that more than one mechanism is involved. In delayed death, chromosome breaks and mutations have been invoked as possible mechanisms. The mechanisms resulting in cellular death may be better understood when the role of repair processes in irradiated cells have been studied, since the ultimate expression of a radiation effect depends not only on initial injury but also on the ability of the cell to repair the injury.[425] Most chromosome breaks rejoin; metabolic and synthetic processes take part in healing,[426] energy from ATP being required.[427,428] Recent experiments by Elkind and Sutton[429] have made it clear that repair operates in mammalian cells and influences the ultimate expression of late effects.

174. A clear distinction should be made between biochemical processes leading to delayed death and those leading to interphase death. In the former, synthesis of nucleic acids and proteins continues.[315] Radiation-induced interphase death is sudden and marked by an arrest of metabolic processes in cells with very wide differences in metabolic behaviour, e.g. cells which are not dividing (lymphocytes), cells dividing infrequently (oöcytes), and cells continually dividing (B spermatogonia).

175. The biochemical causes of interphase death are not understood, but it is possible that Creasey and Stocken's work[403] on nuclear phosphorylation provides a first clue. Their data indicate that nuclear phosphorylation is an extremely radio-sensitive process and is rapidly inhibited. As yet, this process has been detected in nuclei of so-called radio-sensitive tissues only; it has, therefore, been suggested that cells dependent upon this source of energy are those which undergo interphase death at small doses. Creasey and Stocken remark, however, that failure to show nuclear phosphorylation in radio-resistant cells may be due to an increased activity of degradative enzymes rather than to absence of this metabolic process.

176. Nuclear phosphorylation could also be involved in reproductive death if the energy necessary to heal chromosomes was provided by this phosphorylation. A role of mitochrondrial oxydative phosphorylations in interphase and reproductive death cannot be excluded. X-irradiation *in vivo,* in fact, damages mitochondria in liver cells[430–432] even at doses as low as 25 r. Mitochondrial oxydative phosphorylation in plants is immediately and greatly reduced after a single dose of 3,000 r, the effect being more pronounced when cells are irradiated *in vivo* than *in vitro.*[433] Similar effects are also seen in microbial cells.[434]

177. It is difficult to draw a coherent picture of the biochemical basis of cellular death at this time. The possible role of nucleic acids and protein synthesis has been discussed, but much more extensive information is needed on the cytological alterations of sub-cellular structures produced immediately after irradiation. Nor can other biochemical processes affecting permeability,[435, 436] the maintenance of ionic balance[437,438] or the disruption of nuclear and cytoplasmic membranes,[439] be ignored as factors in the mechanism of cellular death.

VII. Biological variables influencing radiation response

Concept of radio-sensitivity

178. Various criteria, e.g. death of cells, inhibition of mitosis, impairment of biochemical and physiological functions, are currently used to determine radio-sensitivity. However, when radio-sensitivities of different types of living organisms are compared, survival after irradiation is usually chosen as the parameter. The selective action of radiation on different parts of the cell and the relations between differentiation, mitotic activity, and radio-sensitivity were described within a decade of the discovery of X-rays. In 1906, Bergonié and Tribondeau[440] formulated the principle that cells in active proliferation are more sensitive to irradiation than non-proliferating cells, and that radio-sensitivity varies inversely with degree of differentiation. Radio-sensitivity depends on various factors, physical (e.g. temperature), chemical (e.g. oxygen tension, hydration), biological (e.g. ploidy, phase in the division cycle in which the cell is irradiated). Radio-sensitivity further depends on the metabolic state of the cell.

Variations in radio-sensitivity with stage of division

179. The different phases of mitotic and meiotic divisions have different sensitivities to radiation. Attempts have been made to link these variations in sensitivity to various phases in the formation of new chromosomes and to the synthesis of nucleic acids during division.

180. Cell survival, gene mutation frequency, and frequency of chromosomal aberrations all respond differently according to when the cell is irradiated. It is difficult to define the most critical moment as it may vary for different cell types and for different lesions.[413,414] Most experimental efforts to clarify this issue have been carried out on germ cells, in particular on both fertilized and unfertilized eggs of several organisms. The end-effects most frequently used as criteria of damage are either survival, or frequency of chromosomal alterations in these cells. It is widely held that variation in sensitivity during division is a general phenomenon and is present in all cells, whatever lesion is taken as the end-point of irradiation.

181. Nevertheless, some recent results suggest that sensitivity of mammalian tissue culture cells to the lethal effect of radiation is independent of the division stage in which the cells are exposed. Survival curves[397,400,441–443] obtained with mammalian somatic cells both *in vivo* and *in vitro* have failed to show the existence of a resistant fraction in cell populations despite the existence of heterogeneity in stage of division. However, experiments with synchronized cultures of HeLa cells have revealed some fluctuations in sensitivity during mitotic division.[35] Cellular morphology does not affect radio-sensitivity of these cells appreciably since the LD_{37} of different cellular strains (epitheliod, fibroblastic, etc.) ranges between 75-166 r only.

Variation of radio-sensitivity with ploidy

182. Ploidy is one of the biological factors affecting cellular radio-sensitivity at the level of the primary radiation injury. The shape of yeast survival curves depends on the ploidy of the strain. Latarjet and Ephrussi[13] showed that survival of haploid strains exposed to X-rays follows a one-hit curve whereas that of diploid cells follows a two-hit curve. These authors, and subsequently Tobias,[444] propounded the hypothesis that inactivation of a haploid cell is caused by a single recessive mutation whereas to inactivate diploid cells two homologous sites must be injured.

183. Extending such studies to higher polyploids, Mortimer found that radio-resistance reaches a maximum for diploid strains and then diminishes with increasing ploidy.[14] Mortimer's results have been confirmed by Magni,[445] but these authors interpret their findings differently. According to Mortimer, haploid strains are mainly inactivated through lethal recessive mutations, whereas with strains of higher ploidy dominant lethal mutations are chiefly responsible for the inactivation. Both types of mutations would be produced in haploid and polyploid strains, the problem being to evaluate quantitative relationships of the two types. Magni suggests that, in addition to recessive and dominant mutations, non-genetic injury accounts for a sizeable fraction of radiation lethality.

184. In some other systems a positive correlation between increasing ploidy and radio-resistance has been seen. Sparrow *et al.*[446,447] found that, on the average, doubling of chromosome number in plants increased radio-resistance by a factor of 1.67. Analogous results were obtained with polyploid cereal seeds[448] and with hyperploid tissue culture cells.[449–451] In contrast, Till[452] found identical dose-effect curves for cell lines with different chromosome numbers and Rhynas and Newcombe[453] have described radiation-resistant cell lines of the *L* strain with a lower number of chromosomes than the radio-sensitive line. Of interest in a consideration of the influence of polyploidy is the inverse relation between nuclear volume and radio-sensitivity in 23 diploid species of plants.[447] The role of ploidy in cellular radio-sensitivity becomes more complex when stage of development is considered. Clark[454] showed that, in *Habrobacon*, diploid female embryos are more sensitive to irradiation than haploid males during the cleavage stage, whereas during larval and pupae stages haploid males are more radio-sensitive. Tul'tseva[455] and Astaurov have found that, during certain stages of development, radio-resistance increases with increasing ploidy in *Bombyx mori* but that tetraploids are more sensitive than diploids at the end of the larval stage.

Genetic control of radio-sensitivity in bacteria

185. A number of mutations causing differences in radio-sensitivity in *E. coli* are known. The increased resistance of strain B/r results from a single mutational step in its parental strain B.[17] Later, Hill discovered and investigated a more radio-sensitive strain, B/s. This strain also differs from strain B by only a single mutational step.[456,457] A stable strain containing about three times as much protein, RNA, and DNA per cell, isolated by Ogg and Zelle[458] after camphor treatments of strain B/r, was about 2.5 times more radio-resistant to ionizing radiations and in addition had a sigmoidal survival curve rather than the exponential survival curve typical of strain B/r. This radiation resistance segregated in a fashion similar to any unselected marker in genetic recombination tests.[459] Adler and Copeland[460] have produced evidence which indicates that radio-sensitivity in *E. coli* K 12 is influenced by at least 4 genes. The approximate locations of the four genes have been determined in genetic recombination tests. In *E. coli* B, Rousch *et al.*[461] have recently found mutations at two different loci which have a cumulative effect in increasing radio-sensitivity. They too have determined the approximate location of these genes in the genetic map by recombination tests. Furthermore, comparative biochemical studies of these two independent mutations show that one leads to loss of the tendency to form filaments, the other to a strong inhibition of growth and of nucleic acid and protein synthesis after radiation or other treatment. Such comparative studies of mutant strains which differ genetically in response, seem especially promising in elucidating the physiological basis of radiation sensitivity and resistance.

VIII. Primary genetic effects of radiation

186. The tremendous headway in the last decade in the analysis of genetic function and genetic material has led to a clearer view of the need for a more full understanding of the mechanisms of radiation mutagenesis. Some problems are related to the already-mentioned macromolecular chromosome structure, others are related more particularly to the function and structure of the genes. Since Muller's discovery in 1927 that radiations are mutagenic, much work has been accomplished, but no complete answer to the mechanisms of radiogenetics has been given. It has been clear from the beginning that genetic effects include visible chromosomal aberrations. On the other hand, many mutations do not involve any abnormalities at the level of the light microscope, and it has become practical to divide radiation genetics into the studies of *point mutation* and of *chromosome damage.*

The genetic material

187. While one of the most important advances in genetics came from the studies of Morgan, who discovered the linear arrangement of genes along the chromosomes from investigations on *Drosophila,* the most important hypothesis advanced in recent years, derived from work on micro-organisms and viruses, is that of the linear arrangement of genes along the DNA double helix.* Recombination studies in bacteriophages, bacteria, and moulds, in combination with the demonstration that the genetic information is effectively carried in the DNA (or in some cases in the RNA), give convincing evidence.[463] Furthermore, the existence of viruses containing single-stranded DNA[464] or of viruses, whose information is coded in single-stranded RNA molecules, indicates that only one of the two strands of a DNA or RNA molecule may carry genetic information. On the other hand, it has also become clearer in recent years that DNA replication probably concerns double-stranded DNA. Even in the one-stranded ϕ X-174 virus, there seems to be a double-stranded stage during replication,[465] although priming of DNA synthesis *in vitro* is much more efficient if the double-stranded molecule has previously been "melted" to single-stranded units.[300]

188. Hypotheses concerning the structural integration of DNA chains into chromosomes must take into account the existing basic proteins and ribonucleic acids which

* For a review of the subject, see references 462 and 463.

are beginning to be thought of as factors stabilizing, regulating or repressing the genetic units.[372,466] These more refined concepts, fairly well established for micro-organisms, will have to be extended to more complex metazoan cells.

189. A big bar to understanding genetic processes in higher organisms is ignorance of chromosome organization at the molecular level. Although the chromosomes from thymus are 90 per cent nucleohistone, plus non-histone protein, RNA and phospholipids,[467] it is not known how these are made up into the chromosome structure seen under the microscope. Electron microscope studies have repeatedly shown strands of 200 Å diameter,[468] but nucleohistone strands are ten times narrower. Urea and versene can dissociate chromosome fibrils or nucleohistones; this indicates the importance of hydrogen bonds and of metal ions (Ca^{++} and Mg^{++}) in holding structures together.[469] The fact that the U V action spectrum for chromosome aberration[470] is similar to that of nucleic acid indicates that nucleic acid may well play a major role in forming the backbone of the chromosome. That this might well be DNA is supported by the fact that lampbrush chromosomes can be broken *in vitro* by deoxyribonuclease but not by ribonucleases or proteases.[471] On the other hand, Ca^{++} and Mg^{++} deficiency is known to induce chromosome breaks and rearrangements in plants[472] and other organisms, which indicates that these metal ions may play a role in chromosome integrity.

Point mutation

190. The definition of the mutagenic event deserves special attention because of the analysis of the genetics of bacteriophage by Benzer.[462] The size of the genetic material (DNA) depends on the test used to study the mutations. According to the genetic test used, Benzer distinguishes three units:

(*a*) The cistron or unit of gene function is what is being studied when phenotypic changes are observed.

(*b*) The muton or unit of mutation is the sequence in nucleotides which has to be altered for a mutation to occur. Benzer has calculated that a muton could consist of no more than a sequence of 4-5 nucleotide pairs in the r II region of phage T4. As the same phenotypic change (loss of an active enzyme, for instance) may be the result of the alteration of many loci, the size of the cistron is difficult to determine precisely but it is much larger, probably of the order of several hundred nucleotide pairs.

(*c*) The recon—or unit of recombination—is what is assayed when recombination tests are made. One altered muton can be made to recover through recombination, as the result of the replacement of *one* or *two* nucleotide pairs which constitute the recon.

191. At present there is no reason to believe that mutation processes in complex organisms are very different from those in micro-organisms; it is becoming increasingly evident that similar concepts will eventually be applied. It has been demonstrated that the mutation leading to sickle cell anaemia in humans results from the substitution of only *one* amino acid by another in one pair of the four peptide chains of the normal haemoglobin molecule; the 2A chains each have one of their glutamic acid residues substituted by a valine residue.[473] This minute error in the protein is likely to be the result of a corresponding error in the DNA code.

192. Studies are being conducted on the amino acid sequence of specific bacterial or bacteriophage proteins like β-galactosidase and alkaline phosphatase; it is hoped that correlations between alterations of DNA obtained by mutagenic agents and protein sequences will throw some light on the problems of genetic coding. The error in DNA, then, would be replicated in a minutely altered "messenger"—RNA carrying specific genetic information to ribosomes assembling activated amino acids in a specific sequence.[350,474] This very much oversimplified picture of the mechanism of phenotypic expression enables one, however, to understand present concepts of mutagenesis and abnormal phenotypic expression.

Radiation-induced mutagenic effects

193. Damage to DNA of cells by radiation cannot be so controlled that mutations can be obtained independently of lethal events. Although all lethal effects of radiation should not be attributed exclusively to effects on DNA, any alteration of DNA is liable to cause death or mutation of the particular cell. So far, the damage caused *in vivo* by ionizing radiation is not precisely known; the absence of damage to purines and pyrimidine in nucleohistones irradiated *in vitro*[475] proves clearly that effects found in nucleotides or pure DNA cannot be extended to the same material *in vivo*. There are indications that DNA from irradiated bacteria has a slightly lower "melting point", suggesting that H-bonds have been weakened. Different elution patterns of DNA from irradiated thymus cells have been obtained;[476] these indicate some change in DNA structure or molecular size. Finally the sequence of a certain number of short nucleotide chains may be changed.[477] UV irradiation of bacteria appears to lead to the dimerization of some of the pyrimidines, but other reactions, such as hydration of pyrimidines, are also probable. More work is needed to follow the new leads given by recent advances in radiation and photochemistry.[99,478,479]

194. DNA could also be altered as result of uptake, through normal metabolic processes, of an X-ray-altered precursor; this is to be expected from work demonstrating the mutagenic activity of certain purine or pyrimidine analogues. On the other hand, Doudney and Haas have postulated that UV alteration of purine and pyrimidine precursors RNA might lead to mutations after having been incorporated into an abnormal RNA.[480]

Oxygen effect

195. Mutation to streptomycin independence, investigated by Anderson[481] is not influenced by changes in oxygen tension, whereas other mutations in the same bacterial strain depend on oxygen tension during irradiation by ionizing radiation.[481–483]

196. Another important point needs clarification. Does radiation induce mutation by affecting DNA directly or is the DNA altered as a result of secondary action? When DNA in the form of transforming principle,[484] or bacteriophage,[485] is irradiated *in vitro* under conditions where indirect effects are presumably reduced to a minimum, there is no oxygen effect. In bacteriophage, DNA appears to be more sensitive to reducing than to oxydizing radicals. This indicates that X-rays do not act primarily on DNA, but that in certain circumstances this molecule is altered as the result of secondary reaction. However, Hutchinson showed that inactivation of DNA in solution becomes oxygen dependent in the presence of cystein.[486]

Chemically-induced mutagenesis

197. Important progress has come from the study of the effect of several chemical mutagens on DNA or RNA and their correlation with lethal and mutagenic activities in viruses and micro-organisms. Both purine or pyrimidine are known to be chemically changed by a variety of mutagens. Nitrous acid is able to remove the amino group of adenine, guanine, and cytosine;[487] formaldehyde can hydroxymethylate amino groups, but its mutagenic activity in *Drosophila* depends on the presence of adenylic acid in the medium which, after alteration, could become incorporated into DNA.[488] Alkylating agents appear[489] to react in many cases with the N-7 of guanine; this could become unstable and be removed from the DNA chain. Glyoxal derivatives appear to affect guanine. Hydroxylamine[490] appears to react chiefly with cytosine; hydrazine, to remove pyrimidine; a low pH treatment,[491] to remove purine. Acridines, like proflavines, are mutagenic; their action is believed to result from fixation of this reagent between two adjacent base pairs, thus increasing their separation. A comparison of the mutagenic effects of these chemicals with that of radiation could be of great value. The linear dose response curves found in several cases of chemical mutagenesis indicate that, as for most radiation-induced mutations, the process involves a single event. In this case the alteration involves a single nitrogen base in one DNA molecule.

Uptake of abnormal precursors

198. A number of base analogues have also been found to be either lethal or mutagenic. Bromouracil (or bromodeoxyuridine) once incorporated into bacteriophage,[492–494] bacteria, and mammalian cells[495, 496] can produce mutations and lead to increased sensitivity to X or UV radiation.[405, 493, 497]

199. 2-amino purine, another mutagen, is believed to be incorporated or to permit the uptake of another base (perhaps adenine) instead of guanine.[498–500]

Comparison between various mutagenic agents

200. When the frequencies of spontaneous and chemically-induced mutations in bacteriophage T_4 are studied, it appears that some regions of the genome mutate much more frequently than others; the same region does not necessarily mutate with comparable frequency after treatment with various mutagens.[462–501] Proflavine seems to induce a pattern of mutations which differs from that produced by base analogues; the patterns produced by base analogues show some differences when compared with the pattern of spontaneous mutations. One must, therefore, suspect the existence of several classes of mutagens; of these, the base analogue class induces a mutation pattern similar to those produced by five bromodeoxyuridine and the proflavine class. Close study of specific chemical mutagens, and their comparison with spontaneous and radiation-induced mutations, will no doubt bring much light on the molecular basis of mutagenesis.

Biochemical aspects of mutation processes

201. From work on mutagenesis of various analogues and UV radiation, it appears very probable that mutation becomes fixed during DNA replication. Examples of bromouracil-induced mutations are pertinent to this hypothesis.[500] If, as postulated by Freese,[502] mutation can result from replacement of one base pair (A-T) by another (G-C) (or *vice-versa*), then a mistake would appear in the DNA chain.

202. In the mutagenic action of bromodeoxyuridine on T_4 phage, the analogue might take the place of 5-hydroxymethylcytosine and pair with guanine (error in pairing); this would lead to the replacement of a guanine-5 hydroxymethylcytosine (G-H) pair by an adenine-thymine pair after three DNA replications. Alternatively, the bromouracil moiety of the analogue might replace thymine during the first replication (error in replication) and pair with guanine at the next. This would lead to the replacement of A-T by G-H after the third replication.[502] Effectively, mutants appear in a culture after the third DNA replication. 2-amino purine could also lead to the replacement of G-C by A-T, and would, like bromodeoxyuridine, on the basis of this hypothesis, be a good agent for back mutating a mutation due to bromouracil incorporation; examples of chemically-induced mutation and back mutation, interpretable in these terms, are now becoming known.

203. However, it is not at all certain that the reversion of a mutation to wild type is necessarily the exact reversal of the forward mutation, and different base pairs might conceivably be involved in the forward and reverse process as postulated by Brenner, Barnett, Crick and Orgel.[503] It is very possible that the hypothesis of Freese is an oversimplification of the facts. A mutation and back mutation with proflavine might result from addition or deletion of a base-pair; this might lead to a much more substantial alteration of the protein, such as a break or an alteration of sequence in the polypeptide chain. With radiation, it is difficult at present to make any hypothesis, but the concepts of chemical mutagenesis will certainly have to be considered in radio-biology when radiation-induced chemical changes in DNA are better known.

204. It had been known for a few years[504] that the frequency of mutants in bacteria increases with cell division. More recently, Witkin has shown that if protein synthesis is inhibited by amino acid starvation or by chloramphenicol, a lower frequency of bacterial mutants is obtained.[505, 506] This suggests that irradiation produces pre-mutational damage which can eventually be lost, or which can become fixed as a result of protein synthesis. In a study of lethal mutations in *Paramecium*, Kimball[507] has shown that loss of premutational damage is probably due to metabolic repair of localized chromosomal lesions. Lieb has recently shown[508] that when DNA synthesis is retarded by treating the cells with chloramphenicol, the increase in mutants, observed when growth is continued after the chloramphenicol "challenge", parallels the increase in DNA; this strongly suggests that the terminal event in this mutational process is DNA synthesis. Much has still to be learned about induced mutagenesis. The role of RNA suggested by Doudney and Haas[480] is not yet clear. However, one important fact emerges: it is possible to inhibit to some extent mutation fixation in micro-organisms by delaying protein or DNA synthesis.

Mutation expression

205. The biochemical processes underlying the synthesis of cell constituents are becoming better known each year. One of the major problems of present-day biochemistry is the way specific enzymes necessary for these synthetic processes become synthesized themselves. Nisman[509] has succeeded in synthesizing *in vitro* an

enzyme of *E. coli*, β-galactosidase, in the presence of ribosomes of these bacteria, a mixture of the four ribonucleoside triphosphates, and the DNA of a strain of *E. coli* possessing the enzyme. The synthesis does not occur with DNA extracted from an inducible but non-induced strain of the same bacteria. Furthermore, Novelli has shown[510] that this synthesis can be inhibited by X- or UV-irradiation, and that restoration can be obtained by adding the genetically competent DNA to the system. These experiments are pertinent to an understanding of radiation-induced mutagenesis and, together with those on chemical mutagenesis, are the first leads to an analysis of mutation processes at the molecular level. Treatment of the genetic material (RNA) of Tobacco mosaic virus with nitrous acid leads, after infection of the plant, to the synthesis of viral protein with only three abnormal amino acids.[511, 512]

206. The problem of mutation expression is therefore one of information transfer from the DNA to the cellular sites of specific synthesis, many of which are cytoplasmic. One major problem concerns the formation of ribosomes; the way in which they receive their information for specific protein synthesis is at present being extensively studied (para. 140).

Chromosome breaks

207. Point mutations in higher organisms probably result from processes similar to those described for micro-organisms, but the complexity of the chromosomes may complicate the process. On the other hand, chromosome aberrations have been thoroughly analysed in various organisms and described at length in many valuable reference papers. Ionizing radiations can induce breakage of chromosomes or chromatids followed by restitution or illegitimate reunions. This may lead to a variety of aberrations[513] which are visible at the first division after irradiation, or in some instances, only after very many cell generations. However, these aberrations often lead to unequal distribution of chromosomes between daughter cells; these usually lead to cell death. Restitution may be at the morphological level only, and a point mutation, probably due to DNA damage may eventually appear.

208. Similar chromosome damage may also occur after UV irradiation,[513] but is less frequent than after ionizing radiation. It may also occur as an effect of alkylating agents[512] or after incorporation of C^{14}- or H^3-thymidine[514, 515] or of bromodeoxyuridine[497] in cellular DNA.

209. Studies of agents influencing chromosome damage have led Wolff[516] to postulate the existence of two types of chromosome breaks: some which rejoin rapidly and which presumably involve linkages through metal ions, and some which are influenced by post-irradiation protein-synthesis and which are believed to involve covalent links.

210. The relative role of direct and indirect mechanisms in chromosome breakage has been partially clarified by comparing the modifying effects of various chemicals with damage due to chemically induced radicals and radiation.[83, 85] The effect of radiation in producing breaks is mainly direct; it certainly is so for dry DNA. Evidence in favour of direct effect on DNA *in vivo* is provided by experiments carried out with bone marrow cells *in vitro*.[517]

Factors influencing the production of chromosome breaks

211. The effect of oxygen on the occurrence of chromosome breaks produced by radiation is complex. On the one hand, anoxia during irradiation reduces the production of breaks;[219] on the other hand, since the rejoining of chromosome fragments is a phenomenon which requires energy, the absence of oxygen diminishes the frequency of rejoining.[518] Probably connected with the oxygen effect is the effect of temperature.[203] The number of breaks increases with a decrease of temperature; this is consistent with the fact that the tension, and therefore the availability of oxygen, is reduced at lower temperatures.

212. Strictly mechanical agents such as centrifugation and ultrasonics, when applied at the moment of irradiation, increase the amount of chromosome breakage. When cells are irradiated with ultra-violet[519] or infra-red rays either prior to or after exposure to ionizing radiation, the frequency of chromosome breaks is reduced in the former case but is raised in the latter. Infra-red irradiation seems to act through changes in metabolical processes.[520, 521]

213. Biological factors also influence sensitivity to chromosomal damage.[522] Cells from different tissues show different sensitivities.[523, 524] On the other hand, the frequency of breaks per unit of radiation depends on the stage of division during which cells are irradiated.[525] The highest frequencies are observed when cells are irradiated during metaphase and anaphase.[526–528] In the meiotic process, the diplotene stage is most sensitive in animals.[529]

Genetic effects of incorporated radio-active substances

214. Radio-isotopes introduced into organisms may be incorporated into critical molecules. Although most effects are due to ionization by the charged particle emitted from the isotope, some may result from disturbance of the molecule by transmutation of the incorporated atom. The new atom not only has different and, in most instances, incompatible bonding characteristics, but also, in transmutation, gives off recoil and excitational energy.

215. Ionization and excitation from the ionizing particle are so large compared with the energy from transmutation that they usually outweigh the importance of transmutation in radiation injury. However, certain isotopes incorporated preferentially in vitally significant molecules could, by transmutation, cause unique effects not accomplished by ionization or excitation from a charged particle. Accumulating evidence, along with theoretical considerations, indicates that transmutation should be considered as a factor in the toxicity of internal emitters. The atomic number of the radio-isotope, its type of decay, the particle emitted, and the energy released, are obviously important in gauging the significance of transmutation.

Possibility of transmutation effect with C^{14}

216. The disintegration by which C^{14} exerts its biological effect is

$$C_6^{14} \rightarrow N_7^{14} + \beta + 0.155 \text{ MeV} \qquad (1)$$

The mean energy of the β-particles is 50 ± 5 keV; thus the reaction gives rise to fast charged particles for which

the RBE of the energy they release is probably 1. Most of the energy of the reaction (1) passes via the kinetic energy of the emitted β-particle into ionization and excitation of the surrounding material; a lesser part appears at the site of the transmutation reaction itself.[530] Because carbon is a part of every organic molecule in living systems, transmutation may significantly affect key molecules, especially those of the genetic apparatus. Indeed, Totter *et al.*[531] have suggested that the mutational consequences of C^{14} transmutations might be comparable in magnitude to those from the associated β-particles. However, according to Pauling,[532] they are unlikely to amount to more than about 10 per cent of the total.

217. Although it is certainly established that P^{32}, when incorporated into the genetic material of a variety of organisms, produces biological effects by transmutation (*E. coli*,[501,533,534,538] bacteriophage,[536,537] *Paramecium*,[538] *Drosophila*[539-541]), the data concerning C^{14} transmutation effects are less plentiful and less consistent. Apelgot and Latarjet, in tests with H^3, P^{32} and C^{14} labelled DNA in *E. coli* B/r found that, whereas the lethal effect with H^3 was due largely to the emitted beta-particle, transmutation was mainly responsible for the effect with P^{32} and C^{14}.[542] Kuzin *et al.*[543] have reported that the efficiency of incorporated C^{14} in producing chromosome breakage in *Vicia faba* is 10-20 times greater than that of external Co^{60} gamma radiation. By contrast, Williams and Scully[544] failed to observe an increased rate of somatic mutations in *Antirrhinum majus* grown in a $C^{14}O_2$ atmosphere as compared to external gamma radiation. The work of McQuade and Friedkin[514] is especially interesting, for despite the fact that no comparisons were attempted with external radiation controls, the frequency of chromosome breakage in *Allium cepa* root tips was about twice as great when the chromosomes were labelled with C^{14} thymidine bearing the C^{14} in the methyl group as was the frequency observed when the C^{14} was in the 2′ position.

Local consequences of transmutation

218. Three processes may cause disturbances at or very near the site of a nuclear transformation in which a β-particle is emitted:

(*a*) Chemical changes; $C \rightarrow N$;

(*b*) Mechanical recoil of the nucleus which emits the β-particle;

(*c*) The production of residual electronic excitation energy due to the non-correspondence of orbital electrons and nucleus following the transmution.[545]

219. These and other features of transmutation reactions of especial biological interests are summarized below.

Properties of certain isotopes relevant to transmutation problems

	C^{14}	P^{32}	P^{33}	S^{35}	H^3
Half-life	5,760 yrs.	14.3 d	25.4 d	87.1 d	12.5 yrs.
Max. β-energy (MeV)	0.155	1.701	0.27	0.167	0.0176
Mean β-energy (MeV)	0.050	0.71	0.093	0.055	0.006
Max. recoil energy (eV)	6.9	77.3	6.0	3.0	3.2
Mean residual excitation energy (eV)	44.5	60.3	60.3	61.7	24.5
Chemical change	C⟶N	P⟶S	P⟶S	S⟶Cl	H⟶He

220. Except for P^{32}, by far the largest part of the energy locally released is the residual electronic excitation of the transmuted atom. This energy and its magnitude closely resemble the corresponding release in a primary or secondary ionizing event by a fast charged particle. The effects of this electronic disequilibrium are therefore qualitatively indistinguishable, except for site, from those of the emitted ionizing particles.

221. In P^{32} decay, the large recoil energy is clearly sufficient to remove the disintegrating atom from the molecule in which it was previously bound, and to carry it into a neighbouring molecule, together with its associated electronic energy.[545] The recoil energies of all of other transmutation reactions summarized above are much lower and are comparable to the relevant covalent binding energies. Moreover, experimentally determined chemical-binding energies are presumably lower than the activation energies for reactions, even if reactions take place by optimal paths in phase space; the isotropically distributed but directional nature of recoil momentum is likely to make a substantial part of it useless in respect of the optimal reaction path. Hence, even though its chemical binding is simultaneously weakened by the change in its chemical nature, it is doubtful whether, in substances of biological interest, atoms undergoing transmutation other than P^{32}, effectively leave the molecule in which they were bound. An interesting possibility, with a transmuted atom that does not detach from a macromolecule, is that conversion of the recoil momentum to vibrational and other kinetic energy of surrounding atoms may suffice to break significant numbers of important hydrogen bonds in these molecules.

222. The most interesting possibilities of C^{14} transmutation lie in the chemical change, $C \rightarrow N$; this may leave a molecule altered rather than destroyed in function, giving rise to a special class of subtle and viable changes in the genetic system different from those induced by the more destructive ionization or excitation. The significance of the possibility of such changes under conditions of uniform contamination is discussed below.

Ionization dose per transmutation under uniform contamination

223. As will be shown below with uniform incorporation, the practical limitation upon the effect of transmutation itself is likely to be dosimetric. Under such conditions, for every transmutation of a C^{14} atom within an important molecule, $\sim 5 \times 10^4$ eV of ionization and excitation energy will also be liberated; this proportionality will only break down when the molecule under consideration is part of a unit of dimension significantly less than the mean range of the C^{14} β-particle ($\sim 30\ \mu$) and isolated from other carbon-containing units by distances significantly greater than the range. If the efficiency of transmutation in causing a certain effect is η_T, and that of the ionization-excitation energy of conventional ionization (34 eV) is η_I, then the fraction

added to the ionization-excitation effect by transmutation is only $6.8 \times 10^{-4}\ \eta\tau/\eta\iota$. This relation suggests at once that, even for high τ, C^{14} transmutation can be significant only when $\eta\iota$ is very small; unfortunately, it is not of much quantitative worth, since appropriate values are not available. The only estimates available for $\eta\tau$ are from P^{32} incorporated into DNA, where $\eta\tau$ is probably 0.01 or lower,[501, 546] although the efficiency with which the DNA molecule is broken may be in the region of 0.1 for a double helix[547] and reach a value close to unity for single-stranded DNA.[464, 548] For the destruction of infectivity of bacteriophage by P^{32} incorporation in DNA, $\eta\tau$ and $\eta\iota$ values are available, and the ratio $\eta\tau/\eta\iota$ is about 10.[536, 549]

224. Mutation does not necessarily consist only of damage of this kind in the DNA molecule. Changes in at least three types of material might cause mutation:

(*a*) The gene code itself, i.e., in the double-helical DNA (in most organisms);

(*b*) Associated stabilizing material such as histone;

(*c*) The machinery (other than the original gene) by which a gene-replica is made, whether or not this machinery at any stage embodies the gene-code itself in a non-DNA physical form.

The P^{32} data presented relates almost solely to the first of these, and even there is limited to events in the backbone of the DNA molecule rather than the nitrogen bases whose sequence presumably determines the information. Four of the carbon atoms of each average nucleotide of DNA are likewise in the backbone, but chemical transmutation of carbon into nitrogen at most of the others—4 or 5 in the nitrogen-base, 1 in deoxyribose linking nitrogen-base to backbone—could conceivably give rise to subtle viable changes unlikely to be duplicated by gross ionization damage or by P^{32} disintegration in the backbone. In bacteriophage, some protein synthesis necessarily precedes DNA synthesis and gene replication after infection.[550, 551] Experiments on inactivation by P^{32} decay suggest the possibility of a stage at which the genetic information itself is carried in a non-P^{32} containing form.[547]

225. In conclusion:

(*a*) From theoretical considerations based on the large ionization-excitation dose per transmutation, the contribution of transmutation to the biological effect would not be expected to be significant under conditions of uniform incorporation of C^{14} unless the efficiency of transmutation in producing the effect is very much greater than that of ionization. Although experimental data are as yet meagre and inconsistent, certain data indicate that C^{14} transmutation may contribute significantly to chromosome breakage;

(*b*) Because the C^{14} recoil energy is low and the energy of electronic rearrangement strongly resembles the usual ionization-excitation energy, such a contribution is most likely to be mediated through the C→ N chemical change;

(*c*) The area in which to seek such a contribution would seem to lie in phenomena brought about with very low efficiency by ionization: probably not in simple damage to the genic material but perhaps in abnormalities in the components of replicative apparatus where ionization-excitation would, in contrast, be more likely to cause total inactivation.

IX. Recovery at the cellular level

226. The concept of "recovery" at the cellular level covers various phenomena with different mechanisms. At least three should be distinguished:

(*a*) Spontaneous recovery of damaged molecules and structures of the cell; this constitutes genuine recovery;

(*b*) Recovery through action of physical or chemical agents immediately or soon after irradiation; this constitutes a kind of "treatment" of the damaged cells;

(*c*) Replacement of damaged molecules or structures by corresponding molecules or structures from undamaged cells. Here there is no recovery but there is a restoration of cell function.

227. The interval between irradiation and the biological expression of the primary damage indicates a complex process and suggests the possibility of interfering with it to promote the repair of injury. Much work deals with phenomena in bacteria and their related bacteriophages using ultra-violet light. Some results have been extended by the use of ionizing radiation. The inclusion of ultra-violet data in this chapter is justified by the similarities and differences found between the action of ultra-violet light and ionizing radiation. These can enlighten several aspects of molecular biology, in particular those associated with the structure, replication, and biological activity of nucleic acids.

228. Restoration is sometimes obtained by destruction of some intermediate compound before the damage is irreversibly established, e.g. photorestoration of ultra-violet damage,[552, 553] restoration by catalase of lysogenic systems treated with ultra-violet,[238, 239, 554] and restoration by ultra-violet light of X-irradiated yeast and bacteria.[555, 556]

229. Photorestoration (restoration by radiations of the range 3,100-5,500 angstroms) is very general and has been verified in a great variety of biological systems. The study of photorestoration of a transforming factor *in vitro* has led to the discovery of an enzyme in yeast and bacteria which is necessary for restoration.[557] Work with this system will soon give valuable information on the mechanisms of ultra-violet inactivation and photorestoration. Recently, Marmur and Grossman[97] have shown that the PR (photorestoration) enzyme is able to reverse induced linking of DNA strands by UV light.

230. Several radio-biologists have attempted to achieve photorestoration after exposure to X-rays. Dulbecco[558] has shown that coliphage T_2, inactivated by X-rays in synthetic medium (predominant indirect effect), cannot be restored by visible light, but that the same phage inactivated in organic medium (predominant direct effect) shows a slight photorestoration. Similar results have been obtained by Watson,[559, 560] with coliphages T_2, T_4, and T_6. In general, however, there is no photorestoration after irradiation with ionizing particles.

231. Some of the lethal damage provoked by UV light in the coliphage T_4 can be repaired by some cellular reactivation mechanism linked to the presence in this phage of the gene μ. This gene determines the difference in ultra-violet sensitivity between coliphages T_2 and T_4. The primary UV lesions are identical in both phage types, but the presence of the μ allele in T_4 (as opposed to the μ allele in T_2) results in reactivation of about 50 per cent of the otherwise lethal damage. Lethal UV damage reactivable by the μ allele action is almost identical to photoreactivable damage.[561]

232. The restoration effect of ultra-violet light subsequent to X-irradiation has been observed by Elkind *et al.* in yeast cells.[555] Ultra-violet light increases the fraction of cells surviving the exposure to X-rays by a factor of 3 or 4. Analogous effects with spores of *Streptomyces aureofaciens* have been reported by Goldat *et al.*[556] In the latter instance, the restoring action of the ultra-violet was observed for both lethal effects and mutation induction.

233. Restoration by catalase of ultra-violet-induced damage[238, 239, 554] is more restricted, as it applies only to lysogenic systems and is linked to the destruction of organic peroxydes formed in these systems during irradiation.

234. The supply of metabolites to micro-organisms which have lost the capacity to synthetize them can be considered as one possible mechanism of recovery; in this case, however, restoration is apparent only, since the intrinsic damage has not been repaired. Restitution would be achieved if there was a possibility of replacing the damaged molecules or sub-cellular units by non-irradiated ones.

235. The phenomenon of cross-reactivation or "marker rescue" was discovered by Luria with the T-even phages (T_2, T_4, T_6). When a bacterium is infected with active and inactivated phages differing from each other in a few of their genetic loci, some genetic markers of the inactivated parents may appear among the progeny resulting from such a mixed infection. These studies were subsequently carried out in great detail by Doermann *et al.*[562, 563] and were extended to the coliphage λ,[564] and to the *Salmonella* phage P_{22}. This phenomenon may be explained by assuming that the UV lesion, while preventing or delaying the reproduction of the whole phage, destroys only a small piece of its genome. The cross-reactivated loci would be those of the undamaged parts of the irradiated phage which would reproduce only after their "rescue" from the injured genome through genetic recombination with the unirradiated parent.[562, 564] After X-irradiation and after decay of incorporated P^{32}, marker rescue has also been observed in the T-even phages[559, 566, 567] and in the *Salmonella* phage P_{22}.[565]

236. A bacterium infected with a single inactivated phage does not yield active virus; but if two or more inactivated virus particles infect a bacterium, active phage may be released.[568] The phenomenon of multiplicity reactivation has been interpreted by Luria as being due to genetic exchange of uninjured parts of the genome of the parental phages. Further studies[569] have not supported some aspects of Luria's original theory of multiplicity reactivation, but recently Harm[570] and Barricelli[571] have amended Luria's theory to reconcile it with the experimental data. Multiplicity reactivation seems to be restricted to certain strains of phages and to certain types of radiation damage. It occurs with the T-even phages and T_5 with high efficiency; it is less effective with T_1, λ and P_{22}, and not at all effective with T_3, T_7 and the *Pyocyanea* phage P_8.[547] Multiplicity reactivation occurs with high efficiency only when the phage-bacterium complex is exposed to irradiation. To explain the different response to X-rays of intracellular and extracellular phage, Weigle and Bertani[572] assumed the occurrence of an "early step" damage connected with DNA injection which prevents the uninjured parts of the irradiated genome from participating in the sequence of events conducive to reactivation. Although it has been reported that no multiplicity reactivation occurs in T_4 phage incorporated by P^{32} decay,[537] a more recent study has detected this phenomenon.[573]

237. The fact that some of the phenomena of recovery of genetic structures are only seen after UV irradiation is, in general, interpreted as being due to the different primary effects which follow UV and X-ray absorption in nucleic acid molecules. It appears that UV radiation primarily damages bases whereas X-rays primarily produce breaks in the DNA backbone.

238. The damage produced by UV light in temperate bacteriophages can be repaired to a certain extent by the host cell.[565, 574–576] It seems that the normal host cells possess a genetic component which is capable of repairing the UV damaged virus. This is explained by Garen and Zinder in terms of genetic homology between the genome of the phage and the genome of the bacteria in lysogenic systems. The homologous part of the bacteria could replace the injured part of the virus genome through a process of genetic recombination. Similar phenomena have been reported with Rous sarcoma virus[190] and with the measles virus[191] in host animal cells.

239. Another phenomenon of host reactivation has been described by Weigle;[192] it applies to temperate and virulent phages. Among the progeny of irradiated phages grown in irradiated bacteria, a certain fraction of plaque-mutants is observed. These mutants are not seen among progeny of the same phage grown in non-irradiated bacteria. This suggests that the phenomena of reactivation and production of mutants are connected.

240. A restoration phenomenon linked to diploidy has been observed by Latarjet and Ephrussi[13] in *Saccharomyces cerevisiae;* after X-irradiation, haploid and diploid cells can undergo a few abortive divisions before dying (delayed death). In diploid cells, however, a restored cell with normal morphologoy may sometimes arise after a few abortive divisions. Repair of radiation damage may occur in diploid yeast cells if they are starved after irradiation.[193]

241. The replacement of damaged macromolecules by intact ones inside cellular structures also offers a possibility of repair. For instance, survival of *E. coli* B/r to irradiation is higher on a synthetic medium enriched with yeast extract than on synthetic medium only.[194] Similar experiments are those of Daniels *et al.*[195–197] with the large multinucleate amœba *Pelomyxa illinoisensis* in which individuals lethally irradiated with ionizing radiation may be restored to reproductive viability by means of fusion with fragments of unirradiated individuals. When the contents of this amœba are stratified by centrifugation, the heavy third containing nuclei are most active in restoring irradiated cells. Some desoxyribonucleotides were reported to have favourable effect on restoration of hematopoietic cells from radiation injury *in vitro* as well as *in vivo*.[198]

242. Restoration of cells can also be obtained by treatments that modify the post-irradiation metabolism of the cells such as temperature, presence of certain nutrients, metabolic inhibitors. This subject, which is related to the variations in the conditions of the cell populations after irradiation, has been extensively reviewed recently by Alper.[199] Characteristically, the results reported indicate that most treatments which reduce the response to irradiation provide an environment which is sub-optimal for growth.

243. Some physiological functions of cells impaired by radiation may also be repaired. At present, knowledge of recovery mechanisms after ionizing radiation is in its

infancy. This subject is of such importance to radio-biology that research on all aspects of the problem should be emphasized.

X. General conclusions

244. The main conclusions of radio-biology in the 1958 report remain valid and will not, in general, be repeated here. However, because of the importance of the threshold problem, it seems prudent to restate the earlier conclusion that "biological effects will follow irradiation, however small its amount". This conclusion, based largely on theoretical considerations and on the exponential character of many dose-effect curves, is supported by new data on the effects in macromolecular solutions, intracellular structures, viruses, bacteria, and other cellular systems.

245. The main development since the last report has been spectacular progress in the study of biological effects at the molecular level. This applies in particular to the genetic material, DNA, and the way in which this substance replicates itself (DNA synthesis) and controls the synthesis of specific proteins transcribing its information to RNA by a triplet code. In the wake of molecular biology, a molecular radio-biology is now developing and, although still in its initial stages, has already provided some important results. Thus, evidence is now coming forward that the most significant radiation effects (inhibition of mitosis, reproductive and interphase death, mutation), at least in a number of instances, are due to primary damage of the genetic material, namely the chromosomes and, in particular, DNA. How these lesions interfere with DNA, RNA, and protein synthesis has already been much clarified; it is expected that studies on cell-free systems *in vitro* now in progress will provide many answers to still open questions.

246. Understanding of radiation damage in nuclear material has been increased by studies of the effects on the physical and chemical properties of macromolecules, especially nucleic acids and nucleoproteins *in vitro* and *in vivo*. The ESR method seems promising for detection and determination of the fate of free radicals produced by radiation in biological materials.

247. New knowledge of the effects on cytoplasmic functions has contributed to an understanding of the problem of radiation damage to cells. Only by taking into account the mutual interaction of damaged structures in the nucleus and cytoplasm can this complex problem be understood.

248. The important role of recovery at the cellular level in determining final radiation effects has been more appreciated, especially the partial reversibility of initial mutational damage in cells of various origins. However, knowledge in this field is fragmentary; further research is needed.

249. Biological effects after incorporation of P^{32}, C^{14}, and H^3 have been studied. It seems that under most conditions, biological effects are due to radiation rather than to transmutation. However, it has been shown that under certain conditions, particularly after P^{32} and C^{14} are incorporated into essential molecules like DNA, transmutation may lead to chromosome breakage.

250. Radio-sensitivity studies have received new stimulus from recent analysis of genetic factors determining radio-sensitivity in bacteria and from investigations of how these genetic factors are metabolically expressed.

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back to first page

ANNEX C

THE HEREDITARY EFFECTS OF RADIATION

CONTENTS

	Paragraphs
I. INTRODUCTION	1-10
II. THE PREVALENCE OF NATURALLY-OCCURRING HEREDITARY DEFECTS AND DISEASES	11-41
Survey of hereditary disabilities	13-37
Category Ia	14-17
Category Ib	18-29
Category II	30-32
Category III	33-35
Category IV	36-37
Role of heredity in premature death	38
Lethal and detrimental equivalents	39-41
III. THE ROLE OF MUTATION IN SUPPORTING THE PREVALENCE OF HEREDITARY DISABILITIES	42-80
Relative genetic fitness	44-52
Natural mutation rates at individual loci in man	53-64
Autosomal dominant traits	58-59
Sex-linked traits	60-62
Autosomal recessive traits	63-64
Natural mutation rates at individual loci in experimental animals	65-66
Naturally-occurring chromosome aberrations in man	67-70
Naturally-occurring chromosome aberrations in experimental organisms	71-72
Factors affecting the frequency of natural mutation	73-80
IV. THE INDUCTION OF MUTATION BY RADIATION	81-120
Factors affecting the frequency of radiation-induced mutation	82-101
Linearity of the dose-effect relationship and absence of a threshold	83
The dose-rate effect	84-87
"Repair" of pre-mutational damage	88-92
Locus specificity	93
Sex and stage of gametogenesis	94-100
Species specificity	101
Induced chromosome aberrations	102-117
Observations on experimental organisms	103-115
Observations on human cells	116-117
Comparability of radiation-induced and naturally-occurring mutations	118-120
V. EFFECTS OBSERVED IN DESCENDANTS OF IRRADIATED POPULATIONS	121-135
Induced mutations in the immediate progeny of irradiated humans	121-125
Induced mutations in the immediate progeny of irradiated mammals	126-130
Polygenic traits	131-135
VI. INTERPRETATION	136-157
Direct approach	137
Indirect approaches	138-157
The prevalence of hereditary diseases and defects	139-140
The role of spontaneous mutation in maintaining the frequency of hereditary disabilities	141-143
Dose-mutation relationship	144-145
The doubling-dose concept in indirect assessments	146-152
Conclusions	153-157
TABLES	
REFERENCES	

I. Introduction

1. In its consideration of the hereditary effects of ionizing radiation upon man this report, as did that of 1958,[1] centres its attention on the possible consequences of the increases in the level of radiation to which human populations are currently exposed.

2. The Committee's 1958 report presented a comprehensive outline of the genetic hazard of ionizing radiation; the available evidence in man and other organisms was reviewed thoroughly and a variety of approaches was used to elucidate the problem. At the same time it was emphasized that current knowledge was insufficient to complete this task with more than partial success.

3. Since that time several significant developments have been made in radiation genetics and in related disciplines. In particular, progress has been very rapid in the area of human cyto-genetics; considerable attention is now being focused on the induction of gross chromosome aberrations as a serious genetic hazard. In addition, remarkable advances have been made through investigations with mice. These have indicated the existence of previously undetected intricacies in the dose-mutation relationship.

4. Developments such as these have been of great help in understanding the basic problems of radiation genetics. At the same time they have re-emphasized its complexities. The present annex gives particular attention to the effect which these recent advances have had on our ability to estimate the extent of hereditary damage which may be induced in populations by ionizing radiation. In stressing current problems, the report does not enumerate but is nevertheless based on a vast amount of information which has been accumulated over many years in the field of radiation genetics. For an account of earlier data and well-established genetic concepts, reference should be made to the previous report. However, to make this annex self-contained, this older information is summarized at relevant places.

5. All organisms are subject to hereditary diseases and defects. In man, estimates of the size of this burden of undesirable traits are based on the frequencies of:

(*a*) Abortions, still births and neonatal deaths;

(*b*) Infertility;

(*c*) Hereditary diseases and defects;

(*d*) Detrimental deviations from normal in continuously varying traits such as intelligence, life-span and resistance to disease.

6. Deleterious genetic traits are a direct consequence of the presence of specific basic faults in the genetic constitution of affected individuals. These faults may be either undesirable alleles or chromosome aberrations. However, the prevalence of deleterious hereditary traits in a population does not, in itself, provide a complete picture of the amount of genetic damage present. In some instances the fault is partially or completely masked in the heterozygote. In other instances, its phenotypic expression is so different in the homozygous and heterozygous states that it is impossible to express the total detriment to populations in simple terms. Furthermore, environment, in the form either of the remainder of the genotype or of external conditions, frequently has a great influence on the manner in which the fault is expressed.

7. There is no doubt that any increase in the frequency of radiation-induced mutation contributes to the burden of undesirable traits. It is equally evident that the evaluation of this contribution must rely upon an understanding of the genetic structure of a population and the environmental forces to which it is exposed. Moreover, the effect of an increase in the amount of genetic damage, from whatever source, must be considered in terms of a time interval; once inflicted on a population the damage may persist through future generations and may be expressed only intermittently and with varying degrees of severity.

8. There are a number of complementary approaches to the problem of estimating the detrimental hereditary effects of an increase in rate of mutation in human populations. Estimates of genetic hazard can be obtained empirically by the observation of irradiated populations. However, information obtained in this way is meagre, and estimates are more often calculated from what is known about the induction of genetic damage by radiation and from a knowledge of the way in which this damage will be expressed. These more indirect approaches require information on:

(*a*) The magnitude of natural genetic damage within a population as ascertained from a knowledge of the role of heredity in morbidity, mortality, and infertility;

(*b*) The role of recurrent natural mutation in maintaining the prevalence of this genetic damage;

(*c*) The qualitative and quantitative relation between a given dose of irradiation and the corresponding increase in mutation rate.

9. Every approach has its own difficulties and limitations. The direct approach is impeded not only by a meagreness of data but also by the absence of proper controls. Furthermore, in man it is quite impractical, through direct observation, to ascertain the spread of damage over what may be many generations. On the other hand, more indirect approaches require a knowledge of the genetic structures of populations and of genetic mechanisms which we do not fully possess at this time.

10. All approaches often make use of investigations with other organisms because the mechanism by which hereditary information is transmitted is basically the same in all forms of life. Experimental observations in a wide variety of organisms can thus provide a working model of the effects of ionizing radiation on man. However, there may be radical differences in genetic structure between populations because this structure is undoubtedly affected by the environmental conditions under which a population exists. Furthermore, many hereditary defects that are slight but nevertheless of importance to humans are not easily recognized in other species. As a consequence, generalizations based on the results of investigations with experimental organisms entail many uncertainties.

II. The prevalence of naturally-occurring hereditary defects and diseases

11. It is generally accepted that there is a genetic component in much, if not all, illness. This component is frequently too small to be detected; in other instances the evidence for its presence is unequivocal. Nevertheless, the role of genetic factors in the health of human populations has not in the past been considered seriously in vital and health statistics. As a consequence, data on the prevalence of hereditary diseases and defects are now largely restricted to that collected by geneticists for special purposes in limited populations from a small number of countries.

12. An assessment of the hereditary defects and diseases with which a population is afflicted does not necessarily provide a measure of the imposed burden of suffering and hardship on the individual, the family, or society. Such evaluations require, among other things, consideration of the development of medical services and of the cultural values in communities.[2]

Survey of hereditary disabilities

13. In the 1958 report, a detailed examination of data accumulated in Northern Ireland over many years led to a figure of about 4 per cent as the incidence of more readily detected hereditary diseases and defects. That survey has been the most comprehensive undertaken to date, and although limited to a single geographical region, it has provided a useful base on which to formulate overall estimates. New information now permits a revision and reclassification of these. For instance, it is now possible to estimate the frequency of chromosome aberrations and to transfer some conditions, such as Down's syndrome (mongolism), to a different category. The estimate of the incidence of congenital malformations has also been increased. The revised values are summarized below. Disabilities are placed in any of four categories. They are classified according to the role which mutation is believed to play in maintaining their frequency. This subject will be discussed in more detail in section III.

Category Ia

14. This includes harmful traits whose mechanism of inheritance is understood and whose prevalence is determined mainly by the frequency of individual gene, or point, mutations.

15. Several hundred traits determined by single gene substitutions have been identified. A majority of the traits, perhaps 70 per cent, are determined by autosomal dominant genes. Approximately 5 per cent are sex-linked recessive traits, and the remaining 25 per cent are determined by the homozygous expression of autosomal recessive genes.

16. The majority of dominant traits are sufficiently mild in their effects to be transmitted through several generations. In contrast, the detrimental recessive traits now recognized in man are very severe in their effects and, with few exceptions, are lethal in the genetic sense. As a result, although about 70 per cent of well-established specific traits are determined by dominant

genes, in perhaps 90 per cent of persons who show monomeric traits, these defects are determined by dominant genes. In terms of gene frequency, however, genes for recessive harmful traits must far outnumber those for dominant harmful traits in a given population. Furthermore, many hundreds of traits are encountered in man for each of which a recessive mode of inheritance is suggested, but each is so uncommon that adequate evidence for this is lacking. It seems likely that many of these traits are in fact the homozygous expressions of recessive genes and that they contribute in total more than any other class to the frequency of detrimental traits in populations.

17. Traits listed in this category are at present estimated to affect about 1 per cent of all live-born.

Category Ib

18. Harmful traits which are determined by cytologically demonstrable chromosome aberrations are included in this category. Their frequency is maintained mainly by recurrent mutation.

19. There is direct evidence that congenital and other physical defects are sometimes due to chromosome aberrations. This important information has been acquired as a consequence of improved techniques in human cytogenetics. Because most research in this area is new, the subject will be considered here in some detail.

20. As with those traits caused by the action of specific alleles, there is often considerable variation in the clinical severity of defects caused by chromosome aberrations. For this reason, all the clinical aspects of some specific defects remain to be described. Different degrees of mosaicism may be partly responsible for this variation in expression. Many associations of physical impairment with chromosome aberrations are now being reported and it must be suspected that some of these associations are due to chance. Reasonably well-established associations are presented in table I, others, necessitating further confirmation, in table II. All the disabilities noted in tables I and II are congenital, but some diseases of somatic origin are known to be associated with chromosome aberration. Two of these are granulocytic chronic leukaemia[3] and Waldenström's macroglobulinaemia.[4] Such diseases are discussed in annex D.

21. The fact that some well known defects occur as a consequence of anomalies in the number of autosomes was discovered in 1959, when it was demonstrated that Down's syndrome is associated with trisomy of one of the small acrocentric chromosomes (number 21 under the Denver Convention).[5,6] There are two other well-established instances of trisomy syndromes. One involves a member of the 17-18 group,[7] the other a member of the 13-15 group.[8] All three kinds of trisomy are associated with mental retardation.

22. Some detrimental traits are attributable to anomalies in the number of sex chromosomes. This was established when it was shown that a condition known as Klinefelter's syndrome can be caused by an XXY constitution.[9] Related clinical symptoms have now been attributed to XXXY,[10] XXXXY[11] and XXYY[12] karyotypes. Turner's syndrome has been associated with an XO constitution.[13] Females with XXX and XXXX karyotypes have also been described.[14,15]

23. Defects attributable to the presence of chromosome rearrangements have also been detected. Some individuals with Down's syndrome are known to have a forty-six chromosome complement in which part of an extra chromosome 21 is translocated to another autosome.[16-18] Other disabilities that have been associated with translocations or other types of aberration are listed in table II.

24. Defective traits caused by chromosome aberrations are sometimes, as might be expected, inherited through successive generations. A chromosome rearrangement which permits Down's syndrome to be transmitted by phenotypically normal females with a translocation in the balanced state has been demonstrated repeatedly.[17-20] Cases have also been reported of translocation-carrying phenotypically normal males whose children exhibit Down's syndrome.[20,21] Other balanced and unbalanced karyotypes have been noted in parental and child generations (table II). There are indications of differential transmission of aberrant chromosomal types in the two sexes.[22]

25. Mental retardation is one of the common consequences of gross chromosomal aberration. Relevant data have been obtained through the procedure of nuclear sexing of buccal mucosa to detect sex-chromosome anomalies. This procedure reveals deeply staining chromatin bodies within nuclei. The number of these Barr bodies per cell is, in general, one fewer than the number of X chromosomes present; the cells of a normal male are chromatin negative, whereas those of a normal female contain one Barr body. In five surveys, the combined frequency of chromatin-positive individuals among males attending special schools for the mentally backward was 8.77/1,000 (29/3,306).[23-27] Five surveys of male inmates of institutions for mental defectives indicated a frequency of 9.51/1,000 (70/7,358) chromatin-positive cases.[28-32] Two surveys of female inmates of institutions for mental defectives showed a combined frequency of 4.46/1,000 (12/2,689) females with double sex-chromatin bodies and one chromatin-negative female.[31] These figures may be compared with those found in the general population (para. 28 below).

26. Sterility is a frequent consequence of chromosome aberration. Males with sex-chromosome abnormalities are almost always sterile.[33] A study of men attending an infertility clinic showed that about 3 per cent of the patients were chromatin-positive.[34] Among sixty-eight women with a presumptive diagnosis of primary amenorrhoea, 28 per cent were found to have sex-chromosome anomalies.[35]

27. Some cases of still birth and abortion are attributable to chromosome aberration. In a survey for sex-chromosome anomalies in still-born children by nuclear sexing, none of fifty-two females was found to be abnormal, but two of forty-nine males were chromatin-positive.[36] In two instances of miscarriage the embryos have been shown to be triploid.[37,38] Here it was possible to culture material from foetal remnants.

28. A general picture of the prevalence of defective traits caused by gross chromosome anomalies is beginning to emerge despite the newness of this field of research. Some specific traits are extremely rare. However, the frequency of Down's syndrome is about 1.5 per 1,000 total births in Europe, North America, and Japan.[39-41] Comparative figures from other parts of the world are rather scanty. Current data on the frequency of sex-chromosome abnormalities have recently been summarized.[33] Cases of Klinefelter's syndrome (XXY), or at least karyotypes containing a Y and more than one

X, are relatively common, whereas cases of Turner's syndrome (XO) are rare. Three surveys by nuclear sexing of buccal mucosa, have been made among consecutive live-born. A frequency of 2.65/1,000 (18/6,801) chromatin-positive males was found in the combined data. Chromosome studies of seven of the anomalous cases showed that four were XY/XXY mosaics and three had an XXY complement. The frequency of abnormal nuclear sex among females was 0.90/1,000 (6/6,642).[36,42,43]

29. It is now estimated that about 1 per cent of all live-born have some harmful trait determined by chromosome aberrations sufficiently gross to be detected by present techniques. Many of these individuals are mosaics. Rather more than half of the aberrations are anomalies in chromosome number. The rest are intra-chromosome changes, translocations or combinations of these with numerical changes. Only a small fraction of these aberrations are transmitted to subsequent generations. It is likely, however, that estimates of the frequency of transmissible chromosome aberrations would be greater with more refined techniques since these aberrations, being less gross, are more difficult to detect.

Category II

30. This category includes developmental malformations whose mechanism of inheritance is ill understood. Environment is influential in the aetiology of these traits. Drugs, certain infections, and radiation are known to be teratogenic at critical stages of organogenesis, and maternal (intra-uterine) environmental factors are also known to have a great influence on prevalence. The role of mutation in maintaining the frequency of these traits has not yet been ascertained. They often show some familial concentration, but this fact does not necessarily prove the existence of a genetic component.

31. Some of these malformations may be caused by chromosome aberrations. However, no cytological evidence of this has been found in many of the more commonly-occurring malformations.[44-46] It is of course possible that chromosome changes too small to be identified by current techniques are responsible. Alternatively, complex genotypes and unusual environments may be causal factors; it has been suggested that a fraction of congenital malformations are caused by an insufficient degree of such heterozygosity as is necessary to ensure normal development.[40,47] However, it is difficult to distinguish between conditions due to individual recessive genes of low penetrance and any that may arise because of a deficiency of heterozygosity at a multiplicity of loci.

32. Many of these traits are detectable at birth. The frequency of live-born so affected is now estimated to be about 1.5 per cent, but is higher if still births are included. At the age of five years, an additional 1 per cent of affected children can be detected.[40,48]

Category III

33. In this category have been placed serious "constitutional" disorders in which the mechanism and contribution of inheritance are ill understood.

34. Included here are mental illnesses such as schizophrenia and manic depressive reactions as well as disorders such as diabetes mellitus, pernicious anaemia and some affecting the thyroid gland.

35. There is general agreement about the existence of a major genetic component in these traits and, on occasion, a simple mode of inheritance has been postulated for some of them. However, their frequency in the face of strong selection and their distribution in families are difficult to reconcile with a monomeric hypothesis. As a consequence, simple modes of inheritance are not usually assumed.[49] Each of these traits is common and prevalent over most of the world. They were collectively estimated in the 1958 report to affect at least 1.5 of all adults, but this estimate is very uncertain.

Category IV

36. This category includes harmful traits which are determined at single loci, but it is highly unlikely that the frequency of the alleles is substantially influenced by mutation.

37. The frequency of these traits tends to be high in localized areas of the world. This high frequency is a consequence of the fact that each of the traits exists as a part of a system of balanced polymorphism; selection pressures maintain the related genotypes in a state of balance. Included in this category are sickle-cell anaemia and thalassaemia. Many other traits, such as fibrocystic disease of the pancreas, probably belong here. On the other hand, a change in environment at some time in the future might remove some traits from the category. Except in certain localized areas in the world, the prevalence of these traits as currently recognized is extremely low. The subject of balanced polymorphism will be discussed in greater detail in a later section (paras. 47-52).

ROLE OF HEREDITY IN PREMATURE DEATH

38. Abortions, still births and neonatal deaths present special problems in a survey of hereditary defects; not only is the frequency of these defects greatly affected by environmental factors, but the role of heredity in their cause is difficult to ascertain because they are not transmitted to the next generation. In consequence, with the exception of those cases known to be caused by gross chromosome aberration, these defects are not considered in categories I-IV. Nevertheless, breeding experiments in animals have shown that simple genetic mechanisms contribute to their incidence. In other instances the additive effects of several genes with slight individual effects may be responsible.

LETHAL AND DETRIMENTAL EQUIVALENTS

39. All the genetic damage within a population is not expressed phenotypically in any one generation. To a large extent, this is because many detrimental traits are partially, if not completely, recessive; complete expression occurs only in the homozygote. The amount of this recessive damage is an important measure of the genetic health of a population. It can be estimated indirectly from a knowledge of the increase in mortality and morbidity observed in the progeny of consanguineous marriages; in these circumstances the hidden genetic damage can be described in terms of lethal and detrimental equivalents. A lethal equivalent has been defined as a group of mutant genes of such number that, if dispersed in different individuals, it will cause one death on the average.[50] This death occurs with homozygosity. In the same manner, genes leading to visible recessive defects can be defined in terms of detrimental equivalents.[51]

40. The procedure outlined above is a powerful tool with which to estimate the amount of recessive genetic damage within a population. However, lethal and detri-

mental equivalents do not represent genes determining any special category of recessive detrimental traits; when expressed phenotypically in the homozygote, the traits may fall in any of the lists of defects in paragraphs 13 to 38. Furthermore, an estimate of the frequency of equivalents does not provide any direct measure of that fraction of genetic damage within a population which is expressed in the heterozygous condition. Nor does a knowledge of the size of the pool of recessive lethal and detrimental genes, by itself, indicate the mechanism by which these genes are maintained in a population.

41. Estimates of lethal equivalents obtained from available surveys are presented in table III. The surveys are of very unequal scope and reliability, the one carried out in Japan being by far the most extensive. In spite of inconsistencies in the results, including some between the two cities in Japan, it seems reasonable to conclude that individuals in human populations carry from two to four lethal equivalents which are expressed, in homozygotes, before the age of twenty to thirty. In addition, each individual carries approximately the same number of detrimental equivalents.

III. The role of mutation in supporting the prevalence of hereditary disabilities

42. Mutation may be broadly defined as any change imposed in the genetic constitution of a cell. In the present annex, mutation is considered in terms of the two fundamental units of heredity, the gene and the chromosome. Natural mutations are generally referred to as spontaneous though in fact it is understood that there are causal factors over which we do not usually have any direct control. One of these factors is undoubtedly naturally-occurring ionizing radiation. Other physical and chemical variations that occur in nature, and the gene complement itself, probably influence mutability.

43. Two mechanisms are involved in maintaining the prevalence of detrimental hereditary traits within a population. One of these is recurrent mutation. The other is direct transmission of the basic genetic faults through successive generations. The role of transmission is generally expressed in terms of genetic fitness of the relevant genotypes, i.e., the number of their progeny which reach maturity. The importance of mutations in human populations cannot be considered independently of genetic fitness because reliable estimates of specific natural mutation rates and of the over-all contribution of mutation to ill health are frequently dependent on accurate information about this fitness.

Relative genetic fitness

44. The relationship between mutation, genetic fitness, and the prevalence of hereditary disabilities is concisely expressed by the principle which holds that each mutation, whether fully lethal or slightly detrimental, will on the average, result in the death of a descendant or in a failure to reproduce.[52, 53] The more genetically unfit of these mutations, as for instance dominant lethals, will be eliminated quickly, and occasionally without provoking any suffering or undue hardship on the population. Mutations which have less drastic effects on fitness will usually be transmitted through many generations and their phenotypic effects will be expressed in correspondingly more descendants.

45. Genetic damage can affect the phenotype of individuals in either the homozygous or heterozygous states. It is known that few dominant diseases and defects are completely dominant and it is becoming increasingly clear that many recessive traits may not be, in fact, completely recessive. This partial dominance can reflect on the genetic fitness of heterozygotes. The effect that even a minor change in heterozygotic fitness may have on the estimated mutation rate required to maintain the frequency of a defect at a constant level can be illustrated with a trait such as phenylketonuria. This trait occurs with a frequency of 25×10^{-6} in the population of England and the genetic fitness of the homozygote is nearly zero.[54] Under the assumption that the heterozygote has the same fitness as the homozygous normal, a mutation rate of 25×10^{-6} per locus per generation is required to maintain the gene at its present level in the population. If, however, the fitness of the heterozygote is 1 per cent, 2 per cent, or 5 per cent lower, as has been suggested, then the corresponding mutation rates would be three, five and eleven times the previously mentioned rates.[50, 51, 53] In contrast, if a slight heterozygous advantage is assumed, a very different estimate is obtained; with only a 0.1 per cent or 0.2 per cent advantage in fitness, the estimated mutation rate would be only 4/5 or 3/5 that of the original rate.[39] With an advantage of 0.5 per cent, mutation would not be required to compensate for the loss of genes due to deleterious homozygotes; in fact, the gene frequency would increase to a higher level.

46. Genetic fitness of heterozygotes cannot be treated as an invariable property of the two alleles under consideration. Rather, fitness can be influenced not only by the remainder of the genotype, as in the intricate situation involved in populations carrying genes for both thalassaemia and glucose-6-phosphate dehydrogenase deficiency,[55] but also by the external environment. For such reasons an individual estimate of fitness may be valid for the immediate future but less valid when applied over several generations.

47. One of the advances in human population genetics has been the discovery of several balanced polymorphic systems (category IV). The term polymorphism, as used here, describes "the occurrence in the same habitat of two or more discontinuous forms of a species maintained by a balance of selective forces, as opposed to maintenance by recurrent mutation".[56] Such systems arise when a gene confers reduced genetic fitness in some circumstances and increased fitness in others. The increase in fitness may be a consequence of a shift in the macro- or micro-environment or it may be a consequence of heterozygosity as contrasted with homozygosity. The role of mutation in supporting the frequency of polymorphic traits is minor. To predict the over-all consequences of an increased mutation rate it is therefore essential to know the extent to which balanced polymorphic systems contribute to the burden of detrimental hereditary traits. It is also essential to know what fraction of new mutants are equivalent to alleles that are already part of a polymorphic system.

48. The existence of balanced polymorphism is suspected when excessively high mutation rates must be postulated to maintain the frequency of a detrimental trait under the assumption that the heterozygote is neutral. An example of heterozygous advantage in genetic fitness is provided by sickle-cell anaemia, a trait which is fatal in the homozygote. The distribution of the sickle-cell trait has been investigated over large areas of the world and is very uneven; the trait is completely absent in a number of populations, yet the homozygote

has a frequency of 3 to 4 per cent in some populations of Asia and Africa.[57] It has now been demonstrated that heterozygous individuals have an increased resistance to malignant tertian malaria and a consequent selective advantage in a malarial environment.[57, 58] It is likely that other serious haemoglobinopathies, including thalassaemia, are maintained by a similar mechanism. Current world-wide measures to eradicate malaria will have the effect of reducing the genetic fitness of heterozygotes. As a consequence, a reduction in gene frequency is to be expected. However, the rate of reduction will be slow and the trait will continue to be carried for many generations. It has been suggested that the inexplicably high frequencies of some detrimental traits are a consequence of relatively greater genetic fitness of heterozygous carriers at some time or place in the past.[59]

49. The frequency with which balanced polymorphic systems occur in human populations has yet to be determined. Relevant to this problem are two contrasting but not mutually exclusive hypotheses that have been proposed for the construction of extreme models of gene behaviour. One has been termed the classical, the other the balance hypothesis.[59] Under the classical hypothesis, it is assumed that genetic variability is maintained by recurrent mutation. Furthermore, it is assumed that almost all mutations are unconditionally deleterious and subject to selective elimination; heterozygous advantage is restricted to a small number of loci although it may contribute greatly to existing genetic variability. The balance hypothesis, on the other hand, assumes that genetic variability is to a large extent maintained by heterozygous advantage; mutation may not be unconditionally deleterious and a certain level of heterozygosity is essential to high fitness.

50. Using the concepts of lethal and detrimental equivalents, it is possible to deduce the relative importance of these two models. It has been calculated that an inbreeding depression of such a high degree as has been detected experimentally cannot be expected from systems of balanced polymorphism; this has led to the conclusion that most hereditary defects revealed by inbreeding are maintained by recurrent mutation.[60] A similar conclusion has also been reached from different evidence; an analysis of the frequencies and modes of inheritance of deafmutism, limb-girdle muscular dystrophy and low grade mental defects has suggested that the mean genetic fitness of a population would be impossibly low if the prevalence of these and other traits were not maintained by mutation.[51] On the other hand, in a recent study of two Japanese populations, the detected inbreeding depression was so slight as to indicate that the role of balanced polymorphic systems in maintaining the prevalence of hereditary effects is greater in those populations than in others previously studied.[61]

51. Investigations with irradiated experimental organisms have also produced conflicting evidence,[62–67] a fact which may well reflect the importance of strain differences and environment in the phenotypic expression of genotypes. It is also possible that a variation in frequency of gross chromosomal aberrations with different doses of radiation contributes to differences between results.

52. In the absence of complete information about the role of balanced polymorphic systems it is usually assumed that most of the genetic damage within populations is mutation-maintained; this avoids the risk of underestimating radiation damage. Even if this assumption is incorrect, it is possible that most new mutant alleles at loci involved in polymorphic systems are unconditionally harmful in contrast to those alleles which support the polymorphic systems in nature. In these circumstances it is important to know the average reduction in fitness of the heterozygote, since this value determines the number of generations over which a temporary increase in mutation rate would be felt by a population. It also determines to some extent the magnitude of the total damage. There is no general information about this value in man. In *Drosophila*, extensive studies have indicated that the average reduction in fitness of heterozygous lethals and semi-lethals is about 2 per cent.[50, 68] It would probably be larger in poor environmental conditions.[69, 70]

Natural mutation rates at individual loci in man

53. The frequency of mutation at a locus can only be studied when the mutation determines a specific detectable trait. Mutation is always an uncommon event; a freshly-arisen specific mutation seldom occurs with a frequency of more than one in fifty thousand gametes. It follows that very large populations must be studied to obtain a reliable estimate of this rate.

54. In many respects man is a very suitable organism for the observation of mutation rates because large free-living populations can be defined and close relatives are easily identified. Furthermore, the high efficiency of medical diagnostic procedures renders relatively easy the identification of many traits in man that might be missed in experimental animals. For these reasons, more estimates of natural mutation rates are available for man than for most species other than micro-organisms. There are, however, difficulties in relating traits to specific mutant alleles in man. These difficulties do not arise as frequently in animals, because planned breeding and genetic analysis can be employed.

55. Some of these problems are specific to dominant, some to sex-linked, some to recessive gene mutations, and some are common to all three. Those common to all three derive from the following circumstances:

(*a*) Certain mutant gene traits are mimicked by phenocopies. These are identical or nearly identical traits determined not by the genotype but by abnormal development in the embryo of foetus *in utero*. However, careful clinical study often serves to distinguish such phenocopies, as for example in the case of certain cataracts, and in cases of congenital deafness;

(*b*) Certain traits which are difficult if not impossible to distinguish clinically, are sometimes determined by mutations on different chromosomes. For example, ichthyosis vulgaris is determined by an autosomal dominant gene and also by a recessive gene on the X-chromosome;

(*c*) Some clinically identical traits seem to be inherited as if they were autosomal dominant at some times and recessive at other times. Examples are achondroplasia and a number of degenerations of the choroid in the eye. This variation may be a consequence of mutations to different alleles at the same locus, of mutations at different loci on the same chromosome, or of mutations at loci on different autosomes;

(*d*) Some traits, though apparently inherited in the same manner, show differences between families which suggest that the causal mutations are different in kind. Although different loci may be involved in these cases, it is conventional to express mutation rate in terms of a

single locus. Such difficulties lead to over-estimates of mutation rates.

56. Precision in the estimation of the mutation rates of genes determining harmful traits in man depends upon the completeness of ascertainment of the character in a large defined population. High precision can only be achieved where the medical and social services for the population are well organized. Even so, complete ascertainment is virtually impossible and can never be assumed as certain. Incompleteness of ascertainment tends to result in under-estimation of mutation frequency.

57. In generalizations of the mutation rates per locus in man one further factor must be considered. If the mutation rate of a gene is very low the trait may arise too infrequently to be recognized as of genetic origin, or even if so recognized, it may not attract study because of the great difficulty of collecting a sufficient number of cases. In consequence, only those traits occurring with a sufficiently high frequency to give a reliable estimate of mutation rate are selected for investigation.

Autosomal dominant traits

58. A direct method is applicable for estimating rates of mutation to dominant traits. This method attempts to identify all cases of a certain trait in the offspring of parents not affected by the trait. If it is assumed that the gene is fully manifested, then each case must represent a mutation in the germ cells of one parent. As each birth results from two gametes, the mutation rate per gamete is one-half the frequency per birth. This method can seldom be employed and can be fallacious if unrecognized phenocopies occur.

59. An indirect method can also be used. This method assumes that an equilibrium has been reached in which the frequency of the trait is more or less constant. At this equilibrium, the number of fresh mutations arising in the population in each generation is approximately balanced by the number of mutations eliminated by selection. The equilibrium equation is $\mu = \frac{1}{2}(1\text{-}f)\,x$, where μ is the mutation rate per gamete per generation, x is the trait frequency in the population, and f is the relative fertility of the individuals bearing the trait. In such an equilibrium the value of f is of great importance. It is, however, difficult to estimate with accuracy. If f is zero then the condition is not recognized as genetic in origin. On the other hand, relative fertility of the affected individuals can be estimated only if it is as low as 85 per cent. As a result, estimates of mutation rate tend to be made for traits with a value of f between 0.0 and 0.8. A number of estimates are listed in table IV.

Sex-linked traits

60. Estimates of the recessive mutation rate at loci on the X-chromosome must be made by an indirect method. The equilibrium equation is $\mu = \frac{1}{3}(1\text{-}f)\,x$. In this case, it is assumed that the fertility of heterozygous females is the same as that of homozygous normal females in the population.

61. The most reliable estimates of mutation rates for a sex-linked recessive gene are those for Duchenne-type muscular dystrophy. However, there is some evidence that even this trait is clinically heterogeneous. In consequence, current estimates may represent the sum of mutations at more than one locus.

62. No reliable estimates of the mutation rate for haemophilia A have been made since haemophilia B (Christmas disease) was identified as a separate entity. The proportion of haemophilia types A and B varies in different countries. Possibly the older estimates of the mutation rate for haemophilia, if reduced by about one-tenth, serve as reasonable estimates for the locus determining haemophilia A. However, the trait can be so mild that ascertainment is almost certainly incomplete. This tends to produce under-estimates of the true mutation rate. Some estimates are presented in table V.

Autosomal recessive traits

63. Only indirect estimates of autosomal recessive mutation rates can be made and these are of very uncertain reliability. The equilibrium equation is $\mu = (1\text{-}f)\,x$. In man, the value of f is zero or extremely low for the great majority of recessive homozygotes. Exceptions are albinism and some forms of recessive deaf-mutism. Even with these conditions, however, the value of f is not over 0.5. If f has a value of zero then the estimate of mutation rate corresponds to the trait frequency. Here, however, there are many difficulties. It is assumed, as for sex-linked genes, that the fertility of the heterozygote is the same as the average in the population. However, a high proportion of all mutant genes in the population are in heterozygotes. For this reason any selection in favour of or against the heterozygote has a much greater effect on the prevalence of a trait at equilibrium than has the loss due to homozygosis. Furthermore, a shift in the environment can upset the population equilibrium by affecting the genetic fitness of the different genotypes. When this happens, many generations may pass before equilibrium is restored. Again, changes in marriage customs can affect the frequency of different genotypes. A decline in the amount of inbreeding has been noted in Europe during the last century or two; such a circumstance is likely to lead to estimates that are too low.[71] Some estimates of autosomal recessive mutation rates are presented in table VI.

64. In spite of all the reservations, there is a large group of grossly harmful mutations, autosomal dominant, recessive, and sex-linked recessive, whose estimated mutation rates cluster around 10×10^{-6} per generation. However, this clustering may be conditioned largely by the selection of traits for study.

Natural mutation rates at individual loci in experimental animals

65. With experimental animals it is possible to estimate natural mutation rates with methods that involve test matings. In the mouse, the rates of natural visible mutation have been estimated at seven loci. These loci are identified by recessive visible alleles namely: *a* (non-agouti), *b* (brown), *c* (chinchilla), *d* (dilution), *p* (pink-eye), *s* (piebald spotting), and *se* (short ear). The loci are distributed on five of the twenty chromosomes. There is linkage between *d* and *se* and between *c* and *p*. These alleles were selected for various radiation studies and should not be considered a random sample. The over-all mean mutation rate is estimated to be about 7.3×10^{-6} per locus per gamete (table X).

66. Estimated values of natural mutation rates at specific loci in *Drosophila* were discussed in the previous report and in a recent review.[72]

Naturally-occurring chromosome aberrations in man

67. Man has a relatively stable karyotype; the diploid chromosome number is forty-six.[73,74] Nevertheless,

with the development of improved techniques in mammalian cytology, examples of aberrations already well known in plants and insects are being accumulated. The detection of chromosome anomalies in man is aided by the relative ease with which associated abnormal phenotypes can be recognized. On the other hand, cytogenetic techniques are not yet far enough advanced to permit the detection of less obvious aberrations. Those which are not now detectable include reciprocal translocations of nearly equal size, inversions and either small duplications or small deletions having a length less than 10 per cent that of the affected chromosome. Other aberrations may be undetected because they are lethal at a very early stage in embryo development.[33]

68. The most common of detected aberrations are trisomies of the smaller autosomes and either monosomy or polysomy of the sex chromosomes. It seems likely that monosomy and trisomy of autosomes other than that producing Down's syndrome, are rare or usually lethal.[75] Triploidy has been detected,[37,38,76] and translocations and other aberrations are frequently reported (tables I and II).

69. Whole-chromosome anomalies may be a consequence of either chromosome loss or "non-disjunction". Monosomy can result from either process, but polysomy is attributable only to non-disjunction. It seems likely that the majority of whole-chromosome aberrations occur in meiotic divisions of a parent or in early cleavage divisions of the zygote. Little is yet known about the relative importance of non-disjunction and chromosome loss during meiosis. However, there is considerable evidence that one or both of these processes frequently occurs in mitotic divisions following fertilization. This evidence is supplied by the existence of mosaics[76-82] and of exceptional twins.[83] The occurrence of whole-chromosome anomalies during mitosis may be more frequent than present data suggest; mosaicism is not likely to be detected when it does not originate in early cleavage divisions. Moreover, selection pressures may eliminate one of the stem lines. The possibility that the processes leading to mosaicism tend to recur in a cell line is suggested by the fact that two or three types of cells are sometimes present in the growth from a single biopsy of bone marrow or even of skin.[84]

70. For one reason or another, most individuals with detrimental traits caused by gross chromosome aberrations fail to produce progeny. Exceptions so far recognized are those phenotypically normal persons with balanced translocations. The general incidence of such translocations is, however, low. As a consequence, the incidence of gross chromosome aberrations in a population tends to correspond with their mutation rate. For estimates of frequency, see paragraphs 28 and 29 above.

Naturally-occurring chromosome aberrations in experimental organisms

71. In the mouse, non-disjunction of sex chromosomes has been shown to occur in meiotic divisions. However, non-disjunction in the first meiotic division is rare in the male and possibly non-existent in the female. In contrast to man, XO karyotypes occur much more frequently than do XXY karyotypes.[85] There is evidence that XO individuals most often result from the loss of the paternal sex chromosome some time between sperm entry into the vitellus and the first cleavage. This evidence is based on the observation that when $X^{M}O$ and $X^{M}X^{P}Y$ mice are scored simultaneously (the superscripts M and P designate maternal and paternal derivations of the X chromosome) the relative frequencies are 0.7 per cent and 0.02 per cent, and on the fact that primary XO's are not randomly distributed.[86,87] Deficiencies and monosomies that would have been detected in extensive experiments on certain genetically marked autosomes in the mouse have so far not been found.[85,88] Spontaneous translocation has been observed in the rat.[89]

72. In *Drosophila*, maternal non-disjunction and meiotic loss of whole chromosomes from dividing cells both operate to produce abnormal eggs. This information has been deduced from the fact that the frequency of eggs with two X chromosomes is less than that of eggs with no X chromosomes. The frequency of abnormal eggs that arise as a result of non-disjunction has been estimated at 0.08 per cent and the frequency of those arising as a result of meiotic loss of the X chromosome at about 0.12 per cent. This produces an XO:XXY ratio of about 4:1.[90] There is also a considerable rate of non-disjunction of sex chromosomes in males; the ratio of scored $X^{M}O$ to $X^{M}X^{P}Y^{P}$ individuals is 2.8:1.[91] Monosomy and trisomy of the small fourth chromosome occurs spontaneously but non-disjunction or loss of the second and third chromosomes has not been detected by genetic or cytological methods of analysis. It is probable that these events occur but that monosomy or trisomy of long autosomes leads to elimination in embryonic stages.[90] An early study showed that aging of females by itself has no effect on the natural rate of non-disjunction, although the frequency of non-disjunction following irradiation of virgin females increases through the first ten days.[92] More recent studies have confirmed that maternal age *per se* has no appreciable effect on the frequency of spontaneous non-disjunction.[93] In view of the recognized increase in frequency of Down's syndrome with advancing maternal age[39] and similar observations on the two other autosomal trisomies,[94] this observation shows the difficulty of comparing natural chromosomal mutation rates of flies and man.

Factors affecting the frequency of natural mutation

73. It has long been observed that the frequencies with which natural mutations are found may vary in different circumstances. This variation provides an opportunity to identify and study individual causal or influencing factors. In man, some of these factors can be detected because a relatively long childhood and reproductive span permit the factors to work over a prolonged period of time.

74. With some hereditary diseases and defects it has been observed that mutant frequency among offspring increases with parental age. Such conditions are epiloia, neurofibromatosis and retinoblastoma. This effect of time suggests a simple dependency of mutation frequency on the accumulated dose of the causal factor. Here, by implication, some cumulative influence is involved.[39] In other conditions, such as Down's syndrome, an increase in mutant frequency accompanies rising maternal age but not rising paternal age. Again, a contrasting situation holds with achondroplasia, where the increase in the occurrence of the anomaly is associated only with rising paternal age. Each of these latter examples suggests the presence of influencing factors which are not common to both sexes. Thus, when paternal but not maternal age affects mutant frequency, a dependence of mutation on frequency of cell division in gametogenesis may be involved.

75. A number of factors are known to affect natural mutation frequency in experimental organisms. One of the most studied of these is sex; the spontaneous mutation rate to sex-linked recessive lethals is apparently lower in females than in males of *Drosophila*.[95, 96] An effect of sex on mutation frequency in the silkworm has been noted. Here locus specificity is a factor; at one locus the frequency of mutation is higher in the male, at another it is lower.[97] In the mouse, the data on seven loci under detailed study provide some indication that mutation frequency is lower in females than in males (table X). Females have yielded one mutant among 98,828 offspring. In contrast, males have yielded thirty-two mutants among 544,897 young. However, in man, a study of mutation to the sex-linked trait, Duchenne-type muscular dystrophy, has provided no evidence of a sex difference.[98]

76. Genetic constitution can also affect the frequency with which naturally-occurring mutations are found. A number of specific genes in *Drosophila* have long been known to modify the natural mutation rate by a factor of ten or more over at least a segment of the entire genome.[99] A difference between two geographical races in the frequency with which sex-linked lethals are produced has been demonstrated.[100] In addition, there is no doubt that the mutation rate varies with different loci. The mutability of a gene is also affected by its position in the chromosome.[327, 328]

77. In man, tendencies towards diverse chromosome aberrations in the same individual and towards familial occurrence of diverse chromosome aberrations have been noted. For example, cases of Down's syndrome (trisomy 21) and Klinefelter's syndrome (XXY) in the same individual have been described.[101–105] Associations of XXY with a translocation between chromosomes 14 and 15[106] and of XXX with trisomy 18[107] have been reported. Trisomy for the 13-15 group and an XO constitution has been noted in two sisters.[108] Trisomy 21 has been reported in the progeny of a female carrying an autosomal translocation.[109] Such clustering of gross chromosome aberrations has led to the suggestion that the cells of some individuals may be labile in this respect,[21] or that the occurrence of a first aberration predisposes the chromosomes of a cell towards a second.[84]

78. There is evidence that natural mutations occur at different rates in cells in different stages of gametogenesis. Relevant investigations in *Drosophila* have recently been reviewed.[72] Some loci are more mutable in the germ line than in the soma, while for others the reverse applies.[110]

79. No doubt other as yet unrecognized influencing factors exist. For instance, a significant increase in the frequency of sex-linked recessive lethal mutations has been reported in each of two strains of *Drosophila* as a consequence of space flight.[111] Similar circumstances are also reported to result in an increased frequency of chromosome anomalies (non-disjunction) in germ cells of *Drosophila*.[112] The intensity of cosmic radiation during flight was insufficient to account for these phenomena, and an influence of some other factors must be suspected.

80. It has been hypothesized that the genetic response of a species to the factors influencing mutation rate is itself modified through selection. This concept presupposes the existence of an optimum mutation rate for survival of a species;[113–115] if the mutation rate is too high the species may be crushed under a heavy mutational load and if it is too low the species may not be able to adapt to environmental changes. This concept has been formulated as a mathematical model by introducing what is called the principle of minimum genetic load.[116] A species must adapt itself to progressive changes in the environment and the ability to do so comes from genetic variation, the ultimate source of which is mutation. The importance of new mutation for the future adaptation of the human species is problematical.

IV. The induction of mutation by radiation

81. For obvious reasons, most of our information on the induction of mutation by radiation comes from experimental organisms. However, there is ample evidence that the mutation process is fundamentally similar in all forms of life and there is no reason to suppose that man is exceptional in this respect.

Factors affecting the frequency of radiation-induced mutation

82. The genetic hazards to populations cannot be determined in the absence of a knowledge of the relationship between frequency of mutation and dose of radiation. It is now well recognized that many factors can influence this relationship. The foundation for our knowledge in this field was laid through investigations with *Drosophila*. More recently, studies with mammals have yielded significant information.

Linearity of the dose-effect relationship and absence of a threshold

83. The assumption of a linear dose-effect relationship down to zero dose, and thus of an absence of threshold for mutagenic effects has been considerably strengthened by the results of investigations with *Drosophila*. Studies of mutations at more than fifty loci which affect minute bristles have indicated that acute doses as low as 5 r have a significant mutagenic effect and that the dose-effect curve is linear from lower to higher doses.[117] A linear relationship in the low dose range down to 5 r has also been found for radiation-induced recessive lethals.[118] However, in germ cell stages such as spermatogonia and oöcytes, where the repair of some of the pre-mutational damage is possible, the effect at low doses may turn out to be somewhat less than expected on a linear basis from the mutation frequency at high doses.[119] A departure from linearity has been found for mutations induced with high doses of acute radiation in mouse spermatogonia. A dose of 1,000 r produced significantly fewer mutations than expected on the basis of linearity with results at lower doses.[120] The view that this effect might be due to cell selection gains some support from the finding that fractionation of the dose gave a higher mutation rate which was consistent with linearity.[121] In *E. coli*, evidence of a linear relationship down to doses as low as 8.5 r has been presented.[122]

The dose-rate effect

84. The rate of delivery of ionizing radiation has now been demonstrated to affect the frequency of mutations induced by a given dose. This has been shown for both mice and insects.

85. In mice, the effect of differences in dose-rate on the frequencies of mutations induced at seven specific loci has been studied.[119, 121, 123–127] It has been observed that (table X):

(*a*) When spermatogonia are exposed to doses of 300-600 r at a rate of 8.5×10^{-3} r/min (90 r/week), the frequency of induced mutations is less by a factor of about four than is the frequency following the same dose delivered at a rate of 90 r/min;

(*b*) There is an even more pronounced dose-rate effect in parallel studies of irradiated oöcytes;

(*c*) The dose-rate effect for spermatogonia is not demonstrably greater when the lower rate of delivery is reduced from 8.5×10^{-3} r/min to 1×10^{-3} r/min;

(*d*) Most of the dose-rate effect in spermatogonia is displayed within the range of 24 r/min and 0.8 r/min, whereas in females the range of effectiveness appears to be greater;

(*e*) As in *Drosophila,* no dose-rate effect is evident in spermatozoa.

86. In *Drosophila* a significant dose-rate effect on lethal mutations in chromosome II has been reported with irradiation of oögonia[128] and spermatogonia.[129] In spermatogonia, a lowering of the intensity from 0.10 r/min to 0.01 r/min at a total dose of 200 r results in a significant reduction in mutation frequency. However, a dose-rate effect for contrasting doses of 2 r/min and 2000 r/min at a total dose of 3,000 r gamma radiation has not been observed. In the silkworm there have been found two different types of dose-rate dependence of mutations affecting egg colour and induced during early larval development.[97] In one type the mutagenic effectiveness of chronic irradiation at 0.15 r/min is lower than that of acute irradiation at 320 r/min, and in the other the mutagenic effectiveness is higher with chronic irradiation than with acute irradiation. The former is observed only in the very young larval stage when primordial cells are prevalent in the gonads, whereas the latter is found when germ cells are irradiated in later stages of development. This latter result, which is opposite to the expected effect of dose rate, may not be a dose-rate effect on the mutation process, for it is suspected that cell selection is reducing the yield of mutants at the high dose rate. In any case, it resembles an effect observed at a high dose rate in the mouse, where a dose of 1,000 r gave fewer mutations than a dose of 600 r.[120] Cell selection was invoked to account for this odd result also. In the chalcid wasp *Dahlbominus* no significant dose-rate effect on mutations affecting eye colour has been found when female larvae receive a total dose of 1,000 r at 1,000 r/min and at 0.17 r/min.[130]

87. Although some of the factors that affect the dose-rate phenomenon have been uncovered, investigation has not yet proceeded far enough to elucidate the mechanism involved. Nevertheless, there is strong evidence that it is the mutation process itself which is affected. Thus, cell selection, which may at times play a role, can, in some specific instances, be eliminated as the causal factor. For example, the effect is observed in those mouse oöcyte follicle stages in which cell-killing by the doses of radiation used is negligible.[119, 121] Furthermore, the amount of spermatogonial killing induced by radiation is approximately constant over a range of dose rates in which the dose-rate effect on mutation is evident.[125, 131, 132] If the mechanism for the dose-rate effect does indeed involve the mutation process itself, then it seems likely that some kind of "repair" of pre-mutational damage must be taking place at the lower dose rates.[119] It has been suggested[133] that many of the mutations observed at the seven loci under study may be a consequence of multi-hit chromosomal aberrations which would be expected to occur with reduced frequency at low dose rates.[134, 135] However, there are several lines of evidence, including the shape of the dose-effect curve, that suggest that, although multi-hit aberrations are easily induced by radiation in mouse spermatozoa, the specific-locus mutations induced in mouse spermatogonia are almost never associated with such multi-hit effects. Most mutations in *Drosophila* spermatogonia also appear not to be a result of multi-hit aberrations. This evidence supports the view that the specific-locus mutations induced in spermatogonia of the mouse are point mutations or extremely small deficiencies,[88, 136, 137] and that it is repair of the pre-mutational damage associated with this type of mutation that is involved in the dose-rate effect.[127] Current investigations in other organisms confirm the existence of processes of natural repair or elimination of pre-mutational (primary) damage at low dose rates. The subject of repair will be discussed in detail in the next section.

"Repair" of pre-mutational damage

88. Studies of a variety of organisms have indicated that the process of induction of mutation is not irreversibly fixed at the time of irradiation, but that there is a limited interval between the absorption of radiation energy and the completion of the mutation process during which, depending on the physiological state of the cells, modification of pre-mutational damage is possible. Repair of broken chromosomes by restitutional unions of the breakage ends has been known for a long time and has been studied in some detail. The subject has recently been reviewed.[138] Though there are some reasons to think that restitution and recovery from pre-mutational damage are separate though analogous phenomena, this distinction has not been established by experimental means.

89. In *Paramecium,* post-irradiation treatments are known to alter the extent of recessive damage from a given radiation exposure, provided they are applied before a certain critical stage has been reached in the subsequent division cycle. Moreover, in cells not receiving post-irradiation treatment, the effect of irradiation is increased the later it is administered prior to that critical stage.[139–141] It was shown earlier that a large fraction of the mutational effect of exposure of bacterial cells to ionizing radiation can be reduced by post-irradiation treatment with chemical reagents in certain circumstances.[142] A similar pattern of results has been observed when investigators have worked with UV instead of ionizing radiation.[143–150] It now appears that all these results are consistent with the hypothesis that the terminal event for fixation of some major part of the potential mutation corresponds to the first post-irradiation replication of DNA.[144, 147, 148, 151]

90. Recent data obtained with *Drosophila* show that modification of pre-mutational damage is possible in spermatids, meiotic stages, and late spermatogonia.[152–157] In cells with peak sensitivity, spermatids and spermatocytes, post-treatment with cyanide following exposure to X-rays at a high dose rate may lead to either an increase or a decrease in radiation-induced mutation frequency. Inhibition of oxidative respiration by means of post-treatment with nitrogen causes an increase in mutation frequency in spermatids, meiotic stages, and spermatogonia. On the other hand, fractionation of a dose given at an intensity of 55 r/sec results in a decrease of the mutation frequency in exactly those stages where cyanide is effective. Inhibition of protein synthesis by means of pre-treatment with either chloramphenicol or ribonuclease leads to a significant reduction in the frequency of mutation in spermatids, and in the case of chloramphenicol

col, in the earlier stages as well. Since a ring-shaped X chromosome has been used in such experiments, the reported changes refer to lethal gene mutations and possibly to small deletions. These results have been explained by assuming that, in analogy to the findings in *Paramecium*, two contrasting processes are involved, one associated with the rate of disappearance of pre-mutational damage, the other with the time or rate required for its fixation.[141] Thus, the enhancement of mutation frequency after post-treatment with nitrogen is thought to result from an inhibition of the metabolic repair process. On the other hand, the reduced mutation frequency observed after pre-treatment with both chloramphenicol and ribonuclease suggests that inhibition of protein synthesis prolongs the time-span available for repair of pre-mutational damage. Although it is not known at present what process is involved in fixation of pre-mutational damage in spermatids, the reported findings suggest a correspondence of repair mechanisms in such widely different organisms as *Drosophila* and *Paramecium*.

91. The interaction of oxygen and X-rays in the production of genetic damage, as detected in the progeny of irradiated males of *Drosophila*, has been studied extensively.[158-165] Dose-fractionation experiments, in which part of the dose is delivered in nitrogen and part in air or oxygen, indicate that X-irradiation destroys a protective oxygen-sensitive system. It has been variously postulated that this system acts to reduce the initial amount of damage and that it acts to increase the amount of repair. The system affects both recessive lethals and chromosome aberrations.

92. Table VII summarizes some of the phenomena and material studied both before and since the drafting of the Committee's 1958 report. The similarity of the effects observed is striking, considering the wide range of organisms observed. From these data it can be concluded that a proportion of radiation-induced mutational or pre-mutational changes are subject to natural repair for a finite but relatively brief period after they occur, and that the natural repair process itself is subject to interference by radiation and by metabolic inhibitors. It is important to determine whether this effect is applicable to man, and if so, the single dose-levels or continuous dose-rates at which the natural repair processes are effective, and the critical period of time and the circumstances under which they act. It is emphasized that probably not all pre-mutational damage is reparable and that a linear dose-mutation relationship independent of dose-rate is to be expected at low doses which do not appreciably affect the repair process.

Locus specificity

93. Both the natural and induced rates of mutation have long been known to vary markedly at different loci in various organisms. This observation has now been firmly established in the mouse.[88,136] Among the seven loci under study, the lowest and highest rates for mutations induced in spermatogonia differ by a factor of thirty. This information is based on 174 mutations induced with doses of 300-1,000 r and high-dose rates. Of these, seventy-one mutations were induced at locus *s*, ninety-nine were induced among the four loci, *b*, *c*, *d* and *p*, and only four were induced at the two loci, *a* and *se*. Ninety-two of the mutations were analysed for viability of the homozygotes. Seventy-one (77 per cent) were lethal prior to maturity and twenty-one were viable. There was some variability among the seven loci in this respect also. All the twelve mutations at the locus *d* and all thirty-eight at the locus *s* were lethal. In contrast, of those at loci *b*, *c* and *p*, twenty out of thirty-eight were viable.

Sex and stage of gametogenesis

94. The frequency of radiation-induced mutations can be influenced both by sex and by stage of gametogenesis. The cell stages of greatest importance in determining radiation hazards to man are the oöcyte and spermatogonial, and the genetic effect of ionizing radiation on these stages of the germ cells of mammals has received considerable attention.[166-168] The most extensive investigations have been concerned with the mouse.

95. Male mice irradiated with doses as high as 1,000 r maintain their fertility briefly, and then undergo a period of sterility. Near-normal fertility is then resumed. The temporary sterility is a consequence of the fact that certain spermatogonial stages are extremely sensitive to irradiation.[169,170] Cells in these stages have an LD_{50} of 20 to 40 r. However, a few of the early type A spermatogonial cells survive high radiation doses; these cells repopulate the germinal epithelium and are responsible for the resumption of fertility of the irradiated animal. The existence of the sterile period aids in distinguishing between genetic effects induced in spermatogonial and post-spermatogonial stages.

96. Irradiation of female mice with doses as low as 50 r can result in permanent sterility after an initial period of post-irradiation fertility. A dose-rate effect on this induced sterility has been detected.[171] The permanency of induced sterility is attributable to the fact that the majority of oöcytes are in early stages of follicular development, and are extremely sensitive to radiation. Since there is no new formation of oöcytes in the adult mouse ovary, sterility sets in when the supply of radioresistant oöcytes in older follicles is exhausted.

97. It has been possible to distinguish two kinds of radiation-induced cell death in different types of germ cells in mouse gonads. Most spermatogonia die immediately after irradiation, while spermatocytes show no response until they reach the meiotic divisions. In both cell types, chromosome damage in the classical sense of aneuploidy can, at most, account for only a small part of the cell loss.[172] A similar situation has been found in the rat-kangaroo.[173] These studies suggest that chromosomal damage is a minor cause of cell death in spermatogonia irradiated with moderate doses. The subject of the radiosensitivity of the gonads is treated more fully in annex D.

98. Peak sensitivity to the induction of dominant lethals and recessive visibles in the mouse has been found in spermatids and spermatocytes[174-178] for the male, and metaphase primary oöcytes for the female.[179] With an acute dose of 300 r of X-rays, the mean frequency for mutations at specific loci following irradiation of post-spermatogonial stages is twice that induced in spermatogonia.[178] It has also been shown that exposure of adult females to an acute dose of 200 r of X-rays results in more mutations than a similar exposure of 17½ day old foetuses. In males the induced-mutation frequency has also been observed to be higher in adults than in foetuses, but the difference is not statistically significant.[175]

99. The ratios of induced mutation frequencies at the seven loci under study in mice differs with irradiation of spermatogonial and post-spermatogonial stages.[88,136] Deficiencies large enough to involve both the *d* and *se* loci (with cross-over value of 0.16 per cent) are common among the mutations induced in post-spermatogonial

cells, but irradiation of spermatogonia yields such deletions only with extremely low frequency, if at all. Such deficiencies are, however, induced in oöcytes. It thus seems that mutations contributed to progeny as a result of spermatogonial irradiation differ systematically from those due to post-spermatogonial and oöcyte irradiation.

100. In *Drosophila*, the influence of sex and stage of gametogenesis in radiation-induced mutations is well documented.[72,180,181,329] The lowest and highest frequencies of induced mutation for a given radiation dose vary by a factor of fifteen. Spermatogonia and oögonia are the least sensitive; oöcytes are somewhat more sensitive than oögonia. In contrast, spermatocytes and spermatids are several times more sensitive than spermatogonia. Spermatozoa vary in sensitivity depending on their stage of maturity. The difference in radio-sensitivity between *Drosophila* sperm and spermatids is attributable both to differences in O_2-tension[164,182-186] and to changes associated with protein synthesis.[153-155]

Species specificity

101. Species differ widely in their genetic sensitivity to radiation. The induced rate of mutation at the seven loci studied in mice is about fifteen times that for a comparable group of loci in *Drosophila*.[187] Comparisons of dominant lethals in mammals and *Drosophila*[188] and of chromosome mutation in plants[189] have likewise indicated the existence of species specificity. Radio-sensitivity in different species of rodents has been determined in terms of the number of chromosome rearrangements in the nuclei of spermatogonia exposed to a low acute dose of 4 r.[190,191] Such measurements are difficult to make because the frequency of chromosome breakage varies greatly in different cell stages, a fact which can lead to the confounding of species and cell-stage differences. Nevertheless, the percentage of cells with rearrangements has been reported to vary from 2.6 in guinea pigs to 0.6 in rats, 0.2 in mice, and 0.1 in rabbits. A comparison of the cytogenetic radio-sensitivity of germ cells of the monkey and mouse at doses from 50 to 400 r has suggested that sensitivity of monkeys is twice that of mice.[168,192]

Induced chromosome aberrations

102. Because some serious hereditary defects in man have recently been found to be associated with chromosome aberrations, the role of ionizing radiation in producing these anomalies will be considered in detail. The fact that radiation can cause extensive chromosome changes has been known for many years; investigations in plants[193] and in animals[90] have been reviewed in detail. Actually, it is not always possible to make a sharp distinction between gene mutation and chromosome aberration. Minute chromosome aberrations often cannot be distinguished from gene mutations. Furthermore, rearrangements of chromosome segments sometimes involve "position effects" in which the phenotypic expression of genes is altered.[85]

Observations on experimental organisms

103. One of the most suitable organisms for studies of induced chromosomal changes is *Drosophila;* in this organism small chromosome changes can be detected cytologically by examination of salivary gland chromosomes. Furthermore, detailed information on the linear sequence of specific loci is available. Although observations made with this organism cannot be used for direct extrapolation to man, they nevertheless serve as a useful guide to those effects which might be expected. They are briefly summarized here.

104. Most of the *Drosophila* information has been obtained through irradiation of spermatozoa. Aberrations are detected in either the first or subsequent generations following irradiation. Cytological as well as genetic techniques can be used for this purpose.

105. Viable aberrations resulting from chromosome breakage include duplications, deficiencies, and intra- or inter-chromosome rearrangements. The ability of individuals with deficiencies or duplications to survive this aneuploidy depends upon the length and genic content of the segments involved. Both duplications and deficiencies upset genic balance, and tend to lower viability and to be transmitted as recessive lethals. Viable intra- and inter-chromosome rearrangements include inversions and transpositions of segments within chromosomes, as well as translocations between chromosomes. These aberrations do not involve aneuploidy, and affected individuals are phenotypically normal if "position effect" is not involved. However, their progeny may be genetically normal, or again contain the balanced rearrangement, or be aneuploid.

106. At low doses, the frequency of individuals with aberrations caused by single breaks tends to increase linearly with dose. In some instances it has been noted that small intercalary deficiencies also increase linearly with dose. The frequency of individuals with aberrations caused by two breaks, such as inversions and translocations, increases more rapidly than the first power of the dose, approaching the second power of the dose at lower levels of treatment.

107. Whole-chromosome aneuploidy in *Drosophila* is also caused by ionizing radiation. The induction of primary non-disjunction was first reported in 1921.[194,195] Using irradiated females of *Drosophila virilis* it has been demonstrated that there is a linear increase in the occurrence of primaryXO males in the dose-range 400-1,200 r, and that the induced rate of occurrence of XO males is approximately 1×10^{-5}/r/egg.[196,197] The rate of occurrence of XO males is approximately fifteen times that of XXY females. The ratio of XO:XXY flies is thus greater than the naturally-occurring ratio which is about 4:1. More recently a similar investigation has been carried out with *Drosophila melanogaster*.[93] With exposure to doses of 600 r, 2,400 r, and 3,600 r, the frequency of non-disjunctional males increased at a rate of approximately $2.5 - 3.0 \times 10^{-5}$/r. Non-disjunctional males were more frequent than non-disjunctional females by about one order of magnitude.

108. In mice, gross chromosomal anomalies are rarely found as a consequence of irradiation of parental premeiotic germ cells. This rarity has sometimes been attributed to failure of transmission rather than to lack of occurrence. However, for at least two types of chromosomal aberrations, reciprocal translocations and deletions, this explanation does not seem to be correct. Translocations induced in post-meiotic stages can be transmitted through subsequent meioses to become heritable traits.[166] Thus, a more likely explanation for the rarity of these aberrations following pre-meiotic irradiation is either that the necessary chromosome breaks do not occur or that the broken parts do not exchange. The same situation exists for deletions. An exhaustive study of what appear to be deletions in the *d-se* region of linkage group II in the mouse has shown that these are produced as a consequence of post-spermatogonial and

oöcyte irradiation, but not of spermatogonial irradiation.[88] Transmission of the induced deletions ranges from poor to normal or near normal. Since transmission is possible, it is apparent that either lack of breakage or rejoining is responsible for the non-appearance of the deletions following spermatogonial irradiation.

109. Some types of chromosomal damage are, however, produced with high frequency by irradiation of spermatogonia. Many abnormal anaphases have been found in spermatogonial cells of monkeys two years after exposure.[198] More recently, cytological evidence of chromosome damage in irradiated spermatocytes has been noted at the first post-irradiation cell division in mice.[172] Those particular types of aberration probably cause cell death before maturation of the gametes. However, a recent report suggests that structural changes induced in pre-meiotic germ cells can occasionally be transmitted to progeny.[199]

110. Data on the induction of whole-chromosome changes in the mouse are at present largely restricted to sex-chromosome changes. Experimental work in this field has developed rapidly in recent years.[85] The availability of useful sex-linked marker genes and improvement in cytological techniques have contributed to this progress. The sex-determining mechanism of man has recently been shown to be much more similar to that of the mouse than it is to that of *Drosophila*.

111. In mice, irradiation of sperm increases the frequency with which paternal sex-chromosomes are lost: 1.3 per cent of progeny suffered such a loss after a dose of 600 r as compared with 0.1 per cent in the control.[200, 201] However, the bulk of spontaneously occurring XO individuals are believed to arise from events following sperm entry into the vitellus.[85, 202, 203] Irradiation of the zygote in the interval between sperm penetration and the first cleavage is particularly effective in inducing loss of a sex chromosome. Thus, 100 r yielded 5 per cent XO individuals as compared with 1 per cent for controls. Both maternal and paternal losses can be induced by radiation, whereas only paternal losses have occurred in the controls. No autosome loss has been detected in these experiments in which four and in some cases five autosomes carried genetic markers. This suggests that such losses, if they occur with an appreciable frequency, are lethal.

112. Extensive investigations of the *in vitro* cytogenetic effects of radiation on mammalian somatic cells have been undertaken. Although from the point of view of heredity the important chromosomes are those of the germ cells, these studies of the radio-sensitivity of somatic cells provide a direct method for determining the effect of radiation on chromosomes. It is to be expected that they will play an important role in the future. Measurements are usually based on the frequencies of aberrations detected at the first post-irradiation cell division because many types of aberration are lost in subsequent divisions. Commonly-used mammals include the Chinese hamster,[204–206] the mouse[207–209] and the monkey.[206]

113. Most of the previously known types of aberrations have been detected in these investigations. Breaks are of the chromatid or chromosome type depending upon whether the chromosomes are effectively double at the time of irradiation. Data on the frequency of breaks are not always in good agreement and it is apparent that one of the influencing factors is the method by which cells are cultured. Nevertheless reproducibility of results is good under standard conditions.

114. As is to be expected, terminal deletions increase linearly with the dose but total breakage occurs more frequently than the first power of the dose.[206] At low doses a measure based on linearity is of practical use but a more accurate measure of damage is the "coefficient of aberration production".[210] Values for chromatid aberrations *in vitro* cultures of epithelioid-type cells of monkeys and Chinese hamsters have been found to be in general agreement with those for *Tradescantia* microspores.[211]

115. With experimental mammals it is possible to compare the *in vitro* and *in vivo* rate of induction of visible chromosome aberrations. Somatic cells cultured *in vitro* frequently have a much higher spontaneous mutation rate than do *in vivo* cells.[211] However, investigations with Chinese hamsters and with monkeys indicate that the radiation-induced aberration rate of epithelioid-type cells cultured *in vitro* is not greatly different from that of rapidly dividing cells *in vivo*.[205, 206]

Observations on human cells

116. No measure of the radiation sensitivity of human germ cells has yet been made. Nor have extensive quantitative measurements been made of chromosomal damage induced in somatic cells of individuals. However, it has been clearly shown that chromosomal aberrations are produced.[46, 212–216] This subject is dealt with in annex D, paragraphs 155 to 158.

117. The effect of ionizing radiation on chromosomes of human cells cultured *in vitro* has received considerable attention in recent years.[206, 217–223] As with experimental mammals, data on the frequency with which breaks occur are not in good agreement. For epithelioid-type cells the observed rate at metaphase is about 0.3/cell/100 r[206, 217] but for "fibroblasts" the rate is about 2/cell/100 r.[218, 220] The frequency of chromosome breaks has been reported to be 0.9/cell/100 r for fibroblast-type cells[220] and 2/cell/100 r for leucocytes in freshly-drawn human blood.[222] The coefficients of aberration production for chromatid breaks in epithelioid-type cells *in vitro* and for chromosome breaks in leucocytes are in remarkably good agreement with those for *Tradescantia* miscrospores and for chromatid breakage in epithelioid-type cells of the monkey and Chinese hamster.[222]

Comparability of radiation-induced and naturally-occurring mutations

118. Mankind has long been exposed to natural radiation and it is to be expected that an increase in the level of exposure would not result in any mutations which have not occurred in the past. Nevertheless, natural radiation is only one of the causes of "spontaneous" mutation and it is therefore possible that there may be differences between the spectra of radiation-induced and naturally-occurring mutations.

119. Evidence concerning the comparability of the two sorts of mutations was presented in the Committee's last report.[224] Most of this information came from studies with lower organisms and suggested that, in general, mutations induced by ionizing radiation are similar in kind to those of natural origin.

120. There is evidence that in *Drosophila* the radiation-induced and natural rates of sex-linked recessive lethal mutations are similarly affected by sex and stage of gametogenesis.[72] Close correspondence between induced and spontaneous mutations is not found, however, in mice.[137] Furthermore, in mice loss of the maternal X chromosome can easily be induced by irradiation but spontaneous maternal loss is very rare.[85] There is also

very good evidence from *E. coli* that the natural mutabilities of loci are sometimes not correlated with their radiation-induced mutabilities.[122]

V. Effects observed in descendants of irradiated populations

Induced mutations in the immediate progeny of irradiated humans

121. Direct observations of the genetic consequences to man of exposure to ionizing radiation are now limited to observations of first-generation offspring. Such surveys can be expected to detect only autosomal dominant or sex-linked gene mutations and chromosome aberrations. Among the difficulties of such inquiries are those of estimating the gonad doses actually received by parents, and the small absolute and relative increases to be expected in the frequency of traits determined by such mutations.

122. In these surveys, the data are usually concerned with such matters as abortion, still birth, neonatal death, congenital malformation, and shifts in the sex-ratio of progeny. Results frequently indicate a detrimental effect of radiation but this is seldom statistically significant. One study detected a significant effect of radiation on the frequency of congenital malformations in the progeny of irradiated individuals but interpretation is hampered by the incomplete response to the questionnaires used.[225] Another similar study failed to show this effect.[226] The most extensive survey was carried out in the Japanese cities of Hiroshima and Nagasaki following the atomic bombings; data were collected on more than 30,000 offspring of irradiated parents and on a comparable control group.[227] Observations were made of still births, neonatal deaths, birth weight and congenital malformations. Analysis of these data failed to detect a significant effect of radiation on either the frequency of early death or congenital malformations. It did, however, detect a significant shift in the sex-ratio of immediate progeny. More recently, an analysis of the same data by an independent investigator has produced statistical significance of radiation effects for some other categories of defects and also for over-all early death of progeny.[228, 229]

123. The comparatively high frequencies of Down's and Klinefelter's syndromes permit the effect of parental irradiation on the incidence of these defects in offspring to be studied with relatively little effort. Three such investigations have already been reported. In one of these the radiation history was obtained of the mothers of eighty-one children with Down's syndrome, ninety-one children with cleft lip and seventy-one children with no defect. A possible association between maternal irradiation and Down's syndrome was indicated.[230] However, results of the other two investigations, one of which involved fifty-one patients with Down's syndrome and fifty-one controls,[231] the other 197 patients and 197 controls,[232] were completely negative.

124. A survey of the incidence of congenital malformations in different regions has indicated that higher incidences are associated with geographical areas with high background radiation.[233] Another survey has reported that the frequency of malformation varies with the geomagnetic latitude to which is related the cosmic-ray energy flux.[234] However, it is difficult to prove that natural radiation is the direct influencing factor.

125. A shift in the proportion of male offspring of irradiated individuals has been considered one of the best available methods for detecting induced genetic damage in humans and for estimating its extent. Six such studies have been reported.[225, 227, 235-238] In interpreting the results, the effect of maternal irradiation is more appropriately considered independently of the effect of paternal irradiation. The effect of maternal irradiation on the proportion of male offspring is summarized in table VIII. A consistent reduction in proportion of male offspring has occurred following maternal irradiation. In terms of the simplest genetic interpretation, this can be attributed to the induction, in irradiated women, of sex-linked recessive mutations having a lethal effect on the foetus. The effect of paternal irradiation is summarized in table IX. These latter data are not amenable to a single interpretation; the proportion of male offspring is apparently increased with higher doses, but, in at least some instances, reduced with low doses. The former effect is interpretable in terms of the induction of dominant sex-linked lethals. However, the validity of such a simple genetic interpretation has been questioned on the grounds that the Y chromosome cannot be considered genetically inert.[239] In addition, the induction of XO and XXY karyotypes may also affect the relative frequency of male and female offspring. Furthermore, explanations based on the assumption that the effect on sex ratio is due to damage to sex chromosomes cannot be accepted without reservation. For instance, the drop in proportion of males which has sometimes been noted could be attributed to autosomal mutations which further increase the existing higher mortality of males. The occasionally erratic control values must also be considered in any interpretation.

Induced mutations in the immediate progeny of irradiated mammals

126. By means of properly controlled experiments it is possible to detect induced dominant mutations in the immediate progeny of irradiated mammals. Current information has been obtained principally from mice. In mammals it is particularly difficult to distinguish between gene mutations and minor chromosomal changes. Reduction in litter size, following irradiation of spermatozoa or oöcytes, is most plausibly explained in terms of the induction of chromosome aberration, although gene mutations may also be involved.

127. Spermatogonial cells and oöcytes are of greatest concern in a consideration of radiation hazards. Oöcytes are not replenished, and it has been shown that there is no significant change in mutation rate with time after irradiation of spermatogonia.[240] Irradiation of spermatogonia has much less effect on litter size than does irradiation of later germ-cell stages. This no doubt reflects a drastic reduction in frequency of gross chromosome aberrations. For instance, individuals with deficiencies involving more than one gene locus are commonly found after irradiation of post-spermatogonial cells but irradiation of spermatogonia yields such deletions only with extremely low frequency, if at all. These aberrations do occur, however, among progeny produced after irradiation of oöcytes.[88, 137]

128. The fact that dominant detrimental mutations are induced and transmitted after irradiation of post-spermatogonial stages has been demonstrated by a shortening of the life span in the offspring of male mice exposed to neutrons.[241] In another study, a significant increase in certain types of skeletal abnormalities was found in the first-generation descendants of irradiated male mice.[242] Evidence that some dominant lethality is transmitted after irradiation of spermatogonia has been provided by

analysis of the cause of litter-size reduction following exposure to 1,200 r.[199] The same data indicate that translocations are occasionally found in progeny following irradiation of spermatogonial cells.

129. The specific-locus method of detecting mutations in mice has yielded further information on the dominance of mutations induced in spermatogonia. About three-quarters of all the induced mutations have been recessive lethals. However, some of these have a visible effect on the heterozygote.[136] In a freely-breeding population these mutations might well produce greater total damage as heterozygotes than as homozygotes.

130. In mice, several studies of the effect of paternal irradiation have not revealed any consistent effect on the sex ratio of offspring.[166, 239, 243] Another comprehensive investigation has shown that although the presence of sex-linked recessive lethals in the second generation progeny of irradiated males can be detected, nevertheless sex-ratio changes do not now provide a reliable method of estimating the genetic hazards of radiation because of the complexity of factors governing this ratio.[244] This complexity has been emphasized by the fact that strain differences in the ratio can be obtained through differential selection for low and high blood pH.[245] In fowl, a significant decrease in the frequency of female progeny resulting after exposure of male birds to 600 r has been noted.[246] In *Drosophila*, most investigations have demonstrated some tendency toward an excess of males among the progeny of irradiated males.[247, 248] A significant shift in this direction has been reported recently.[249] Research on sex-ratio shifts needs to be continued in the hope of laying a firm foundation for the application of this method in analyses of radiation-induced mutation in man.

Polygenic traits

131. The subject of polygenic traits was treated at some length in the 1958 report with special reference to intelligence, life span and birth weight.[250] Attention was drawn to the paucity of information regarding the inheritance of continuously varying, or quantitative, traits. These traits, which are influenced to varying degrees by many genes, present a special problem in the estimation of genetic hazards of ionizing radiation to populations. For example, intelligence is influenced by certain rare genes having major effects and by a multiplicity of genes, each with a small effect. In those instances where a mutation has a drastic effect on the trait, or concomitant effects on some other trait, it is individually identifiable and classed as a qualitative mutation. Mutations resulting in such conditions as phenylketonuria and mongolism belong to this category. Where the effect is less drastic no such identification is possible. Furthermore, the frequency of mutations having minor effects is many times greater than is the frequency of mutations having major effects. Finally, a great deal of genetic variability within these traits is common in a normal population, and phenotype is, in addition, often strongly influenced by the environment. In such circumstances the relative contributions of heredity and environment to the over-all phenotypic variability are difficult to determine. A few traits, such as dermal-ridge count, are relatively unaffected by environment after birth; here a more accurate genetic analysis can be made.[251] However, the role of mutation in supporting the genetic variability of polygenic traits has defied any simple analysis.

132. Because rates of mutation of the individual genes in a polygenic system cannot be studied, most investigators have adopted the procedure of expressing induced mutation in terms of the resulting increase in the genetic component of the variance, with or without reference to the genetic component observed in natural outbred populations. The extent of this increase has, in general, been measured either directly by variance analysis or indirectly by calculation of the capacity of an irradiated population to respond to selection. Pertinent information from experiments concerned with natural and radiation-induced mutation rates is summarized in table XI.

133. Such experiments are of special value in indicating whether the genes determining polygenic traits differ in their pattern of mutability from those individually recognized through discrete changes. Estimates of doubling dose for abdominal and sternopleural bristles in *Drosophila* agree well with those for major genes.[252] On the other hand, the estimated induced rates for polygenes controlling viability are high.[253]

134. Loss of genetic variance per generation in an unselected, random-mating *Drosophila* population of limited size is only a small portion of the natural variability of the species. Polygenic traits are evidently well buffered against the effects of mutation. Thus the radiation damage from an increased rate of polygene mutation, although possibly considerable when summed over many generations, is probably small in its impact on the first few generations. Variability in these traits may be maintained in part by a balance of selective forces, a possibility which further complicates the estimation of radiation-induced mutational damage to polygenic systems in an organism such as man, that cannot be directly experimented on.

135. The learning ability, as measured by a maze test, of a population of rats which were irradiated in each generation has decreased in preliminary experiments.[254] If further experiments exclude other interpretations, these results will support the view that radiation results in the induction of many small but deleterious mutations. Again, a significant accumulation of recessive or sub-lethal mutations affecting ability to survive irradiation has been reported in mice after ten generations of chronic gamma irradiation.[255]

VI. Interpretation

136. The preceding sections of this annex were concerned with the genetic concepts and information now available for estimating the hereditary effects of an increase in the level of ionizing radiation. The present section considers the practical problems involved in formulating reliable estimates from this knowledge.

Direct approach

137. An estimate of the genetic hazards of radiation to man can, in principle, be obtained by a direct comparison of the descendants of irradiated with those of control populations. To be reliable, such surveys must be extensive, since most severe genetic defects tend to be rare. Furthermore, many aspects of genetic well-being must be considered and it is desirable to continue the observations over many generations. These conditions have not been fulfilled in any study to date. All surveys made so far have, in addition, been hampered by problems of dosimetry and the difficulty of obtaining proper controls. In the most extensive of these, that dealing with the populations of Hiroshima and Nagasaki, the investi-

gators were unable to detect a significant effect of radiation on either the frequency of early death or the occurrence of malformations. At least, this negative finding suggests that the human genetic mechanism is not substantially more sensitive to radiation than are those of other organisms that have been investigated. It has been suggested that the acute dose required to double the frequency of mutations causing the defects under study is probably more than 10 r.[227] The Japanese survey detected, as did others of lesser scope, a shift in the proportion of first generation male offspring suggestive of the induction of sex-linked lethal damage in irradiated parents. The precise nature of this damage is not known at present.

Indirect approaches

138. Indirect approaches attempt to predict the genetic consequences of exposure to ionizing radiations through an understanding of basic genetic mechanisms and their reaction to radiation. More specifically, estimates are derived through a knowledge of the prevalence of naturally-occurring hereditary ill health within a population, the role of mutation in supporting this burden, and the relation between the dose of radiation and the mutation rate in man.

The prevalence of hereditary diseases and defects

139. There is probably a genetic component in the aetiology of most diseases. It is now estimated that about 6 per cent of all live-born suffer at some time during their lives from serious disabilities in which this component is either known or suspected to be of major importance. Without doubt the estimate of natural genetic burden will increase with future research. In about one third of these disabilities, those of categories Ia, Ib, and IV, the genetic component is high and the underlying genetic mechanism is understood. Of these defects, about half are associated with what appear to be specific alleles, and about half are associated with gross chromosome anomalies. For the remainder of the defects, the developmental malformations and serious constitutional disorders of categories II and III, neither the size of the genetic component nor its underlying genetic mechanism is known with any assurance. These disabilities are almost certainly heterogeneous in aetiology; some are probably almost completely environmental in origin, but in others genotype may be an important factor. However, even where the importance of genetic constitution is suspected, the basic nature of the fault is not clear; complex constellations of genes, specific alleles of low penetrance, or cytologically undetected chromosome aberrations may be responsible.

140. The amount of recessive damage, that hidden in heterozygotes, has been estimated at 2-4 lethal equivalents and an equal number of detrimental equivalents per individual. When exposed by homozygosis, the lethal equivalents are expressed as an increase in miscarriages, still births and in neonatal, infant, and juvenile deaths. The detrimental equivalents are associated with viable malformations and overlap the previous listing to some extent. A comparable measure of genes producing recessive infertility has not been made. No similar method is yet available for estimating the amount of dominant genetic damage within populations.

The role of spontaneous mutation in maintaining the frequency of hereditary disabilities

141. Various mechanisms by which detrimental traits can be maintained in a population are well recognized. A gene sometimes conferring reduced fitness, but never conferring increased fitness, must be maintained entirely by recurrent mutation. On the other hand, if a gene confers increased selective advantage in some circumstances, mutation may have only a minor influence on its frequency.

142. The extent to which such balanced selective forces are responsible for maintaining hereditary disabilities in human populations is unknown at present. Of the defects listed in section II, only among the specific disabilities now recognized to have a high genetic component is it possible to discriminate between those that are mutation-maintained (categories Ia and Ib) and those that are maintained by a balance of selective forces (category IV). At present, traits of category IV provide but a small fraction of the total amount of serious ill health of known or suspected genetic origin. Suitably designed studies will undoubtedly produce more examples in the future. In the meantime, estimates of the importance of balanced selective forces are dependent on the use of indirect procedures or are based on concepts of the genetic structure of human populations which have yet to be confirmed.

143. When the prevalence of defective traits is maintained by recurrent mutation, the genetic hazards of radiation can be estimated if the factor by which mutation rate will be increased by a given radiation dose is known. However, when the frequency of induced mutations has been determined at only one dose it is necessary to know the spontaneous rates to estimate the hazard. Reliable estimates of spontaneous rates can be made only when the genetic fitness of both homozygote and heterozygote is known. It is possible to measure fitness where dominant traits are concerned. However, with recessive traits it is difficult to determine genetic fitness of heterozygotes; as a consequence, reliable estimates of natural mutation rates are rare. Point mutation rates so far estimated cluster around the value 10×10^{-6} per locus per generation. The total mutation rate for gross chromosomal aberrations is now estimated at about 1 per cent per generation.

Dose-mutation relationship

144. The genetic effects of ionizing radiations cannot be understood without establishing a firm relationship between frequency of induced mutation and the dose of irradiation delivered. Most of the earlier information about this relationship was accumulated from the results of experiments with *Drosophila* sperm. Past research led to the working assumptions that: (*a*) the dose-mutation curve is linear in the low-dose range, (*b*) there is no threshold dose, and (*c*) mutation frequency is not dependent on dose rate over the range under consideration. Much effort has been put into the task of either confirming or disproving these three assumptions. Recent investigations have strengthened the first two, but have disproved the last. It has now been conclusively demonstrated that rate of delivery of radiation can have an effect on the frequency with which mutations are found. In male mice, low dose-rates of ionizing radiation produce one fourth as many mutations as do high dose-rates. In females, this phenomenon is even more pronounced.

145. Recent research has increasingly emphasized the fact that radiation-induced mutation frequency can be drastically affected by circumstances other than dose and dose rates:

(*a*) Radiation-induced mutation rates may vary for genes in the same species and this variation need not

correspond to the variation in natural rates. In mice the induced rates per unit dose in spermatogonia at seven specified loci may vary by a factor of thirty.

(*b*) Rate of radiation-induced mutation per unit dose varies in different species. Furthermore, it has been reported that the frequency of cytologically observed induced chromosome abnormalities in spermatogonia of the guinea pig is nearly thirty times that of the rabbit, a closely related species.

(*c*) It is clearly established that sex and stage of gametogenesis can have a profound influence on both spontaneous and radiation-induced mutation frequencies. The existence of such interactions between radiation effect and the circumstances of its delivery add to the complications of estimating radiation effects in humans. For example, it increases the possibility that errors may be involved in extrapolating from one species to another, from non-gonadal tissues to germ cells, and from one germ-cell stage to another.

The doubling-dose concept in indirect assessments

146. The indirect methods for assessing the hereditary effects of an increase in level of ionizing radiation to which a population is exposed involve the estimation of "doubling dose" and the assumption of linearity of the dose-effect relationship. The doubling dose for a particular mutation is that dose which will increase the mutation rate to double the spontaneous rate. A prediction of the phenotypic effect of an increase in mutation rate can be calculated from the fact that the number of affected persons arising as a consequence of a doubling dose delivered in one generation, is equal to the number of affected persons normally present in any one generation as a result of recurrent mutations of natural origin. This increase in affected individuals will be spread over one or more generations depending on the genetic fitness which specific mutations confer on their carriers. The genetic fitness of the heterozygote is of more importance than that of the homozygote in most cases, because rare mutant genes occur much more frequently in the heterozygous state in a random-breeding population. When genetic fitness of the heterozygote is very low, most of the impact of the new mutations will be felt in the subsequent generation; if fitness is reduced by one-fifth, most of the effect will appear within the first five generations; if reduction in fitness is slight the effect will spread over very many generations. A permanent doubling of the mutation rate eventually results in a permanent doubling of the incidence of those traits normally maintained by recurrent spontaneous mutation. On the assumption of an average reduction of 2 per cent in genetic fitness of heterozygotes, most of the impact of a permanent doubling of mutation rate would be felt in about fifty generations. Where systems of balanced polymorphism are in force, natural mutation is a relatively minor factor in the maintenance of genetic variability and a doubling of the mutation rate would have little effect on the prevalence of the associated traits.

147. The usefulness of the doubling-dose procedure was considered in detail in the 1958 report of the Committee. To a large extent this usefulness stems from the fact that whole classes of mutation can be handled as a unit in the absence of any information about the number of loci involved or their individual mutation rates. Tentative numerical estimates of the doubling dose for man were presented in the 1958 report. It was pointed out at that time that little direct information was available on the sensitivity of human genetic loci to radiation. Estimates of doubling dose were consequently based on several other considerations. These included a simple genetic interpretation of sex-ratio changes in man based on the assumed induction of sex-linked dominant and recessive mutations having a lethal effect *in utero*. Account was also taken of the investigation of seven specific loci in mice and of extensive observations on sex-linked lethal mutation in *Drosophila*. As expected, advances in our knowledge have indicated that this estimate is in need of revision.

148. The usefulness of sex-ratio changes in estimating a doubling dose must be considered doubtful because of inconsistencies in the sex-ratio change in the progeny of irradiated fathers (table IX). Furthermore, there is no significant effect on the sex-ratio in the progeny of irradiated male mice.

149. Recently acquired information has also stressed the fact that, apart from the radiation dose alone, there are a number of specific factors which should be taken into account in calculating the doubling dose. Dose rate, sex, and stage of gametogenesis are all factors which affect the frequency and quality of mutation in both mice and *Drosophila* and it must be suspected that they are effective in man. An example of the influence of rate of dose on the calculated doubling dose can be obtained from table X where the main results of irradiation of spermatogonia and oöcytes of the mouse have been summarized. The most important single comparison is that for males between the dose rates of 80-90 r/min and 8.5×10^{-3} r/min. The former rate provides a doubling dose of 30-40 rad, the latter 100-200 rad. A significant dose-rate effect is also evident for oöcytes, and the doubling doses for acute and chronic irradiation show an even greater spread than in males.

150. It is becoming increasingly evident that the spectrum of mutations in man is too wide to be included in a single category for the purpose of estimating a meaningful representative doubling dose. For instance, the doubling dose for gross chromosome mutations may well differ drastically from that for point mutations. If so, the frequency-distribution of hereditary defects resulting from a specific increase in the level of exposure to radiation would not be parallel to the natural spectrum.

151. In view of the undoubted complexities of the dose-mutation relationship, it is evident that this method of assessing hereditary effects of ionizing radiation can easily yield imprecise estimates. At the same time it is equally evident that none of these recently discovered complexities invalidates the doubling-dose concept itself; they merely emphasize that the method must be applied under carefully defined conditions if accurate estimates are to be obtained. In particular, it is important to discriminate between the genetic hazards of chronic low-level exposures and more acute medical and accidental exposures.

152. The difficulties of obtaining information on the hazards of ionizing radiation would be reduced if the large amount of data collected in other organisms could be applied directly to humans. Differences in species introduce into this procedure uncertainties the extent of which is difficult to estimate. A second approach is through the observation of human cells grown in tissue culture; reproducible results relating to radio-sensitivity of cells can be obtained in this way. However, here also extrapolation of information is at present associated with uncertainties. Nevertheless it is clear that *in vivo* and *in vitro* research in different organisms will ultimately

provide a valuable source of information. Such investigations must be accompanied by an understanding of the genetic structure of human populations and the respective roles of mutation and selection in moulding that structure.

Conclusions

153. Sufficient information is not now available to calculate with a useful degree of accuracy a representative dose which would double the mutation rate (doubling dose). Nor is it yet possible to predict directly the quantitative or qualitative effects of such a dose on populations. Nevertheless, information regarding some aspects of the genetic hazards of ionizing radiation can be obtained by the doubling-dose method. This involves the calculation of separate doubling doses for different dose rates, and, in addition, for different specific categories of defects. The complexity of the calculations is reduced by the fact that differential sensitivity of germ-cell stages within each sex can be largely ignored; as far as the genetic hazards of radiation to man are concerned, the significant germ-cell stages are the spermatogonia and the oöcyte. This is true whether irradiation is chronic or acute.

154. The group of disabilities to which the doubling dose can, at present, be most usefully applied are those severe defects maintained by recurrent point mutation (category Ia). Calculations of the 1958 report suggested that the over-all representative doubling dose for man might well lie between 10 and 100 rad, with 30 rad as the most probable value. This estimate was based on studies which involved acute irradiation and the production of point mutations. In the absence of better evidence, the doubling dose for acute irradiation of males does not require revision. However, there is evidence that this value is lower in females; experiments with mice have shown that oöcytes are somewhat more sensitive to acute (but not to chronic) irradiation than spermatogonia. The doubling dose for the two sexes combined must therefore be lower than that for males and may well be about half this value. For chronic irradiation of males, new information from mouse experiments suggests that the doubling dose is about four times the 1958 value of 30 rad. For chronic low intensity irradiation of females, mutation rates seem to be lower than in males. The combined doubling dose for both sexes cannot exceed twice the value for males and is not likely to be much lower than that value. For these estimates, uncertainty due to species extrapolation and the limited number of loci used in experimental studies probably does not exceed threefold in either direction. A permanent doubling of the mutation rate would ultimately double the prevalence of the serious defects under consideration. These are now estimated to have a prevalence at about 1 per cent.

155. The doubling dose for the defects of category Ib, those due to gross chromosome aberration, cannot now be estimated for lack of data. However, the effect of radiation on the frequency of gross chromosome mutation is amenable to study, and it can be expected that continued research in this field will enable estimates to be made in the near future. A doubling of the mutation rate in one generation would almost certainly double the prevalence of these defects in the next generation. This prevalence is now estimated to be about 1 per cent.

156. It is not possible to estimate the doubling dose for the genetic changes contributing to developmental malformations and serious constitutional disorders of categories II and III. The prevalence of these defects might be doubled by a doubling dose but the increase would probably be much less; environment is suspected to have a strong influence on their aetiology, and unrecognized balancing selective mechanisms may also be effective in maintaining their frequency.

157. Significant progress towards an understanding of the genetic effects of ionizing radiation has been made in the last four years. The Committee emphasizes that: (*a*) all research has confirmed the fact that ionizing radiation produces genetic damage at all doses and dose rates so far tested, and (*b*) further progress in understanding the genetic hazards of radiation will come not only from *ad hoc* research in radiation genetics but from an increase in all types of genetic research in man and in experimental organisms.

TABLE I. CHROMOSOME ABERRATIONS ESTABLISHED IN MAN

	Associated clinical condition	*Chromosome complement*	*Chromosome number*	*First reference*
I.	*Anomalies related to chromosome number*			
	Down's syndrome (mongolism)	Autosomes: Trisomy-21	47	6
	Complex congenital malformations	Trisomy-(17–18)	47	7
	Complex congenital malformations	Trisomy-(13–15)	47	8
	Klinefelter's syndrome	Sex-chromosomes: XXY	47	9
	Klinefelter's syndrome	XXXY	48	10
	Klinefelter's syndrome	XXXXY	49	11
	Turner's syndrome	XO	45	13
	Mild mental defect	XXX	47	14
	Mental defect	XXXX	48	15
II.	*Structural anomalies*			
	Down's syndrome with trisomy-21	21 ~ (13–15)	46	16

TABLE II. DISABILITIES WHICH HAVE BEEN ASSOCIATED WITH ABNORMAL KARYOTYPES, EXCLUDING KNOWN MOSAICS

	Clinical condition	*Chromosome complement*	*Chromosome number*	*Reference*
I.	*Anomalies related to chromosome number*			
	Klinefelter's syndrome	XXYY	48	12
	Klinefelter's-Down's syndrome	XXY, trisomy 21	48	102
	Prenatal death	Triploidy	69	37
	Mental retardation	Trisomy 6(?)	47	256
	Facial anomalies	Trisomy 22(?)	47	257
II.	*Structural anomalies*			
	Polydysspondyly	22 ~ (13–15)	45	258
	Familial mental and speech defect	22 ~ (13–15)	45	109
	Primary amenorrhoea	X + partly deleted X	46	259
	Down's syndrome	21 ~ 22	46	18
	Down's syndrome	21 ~ 21, or trisomy 19 and monosomy 21	46	21
	Convulsive disorder	(1–2) ~ (6–12)	46	260
	Klinefelter's syndrome	XXY and 14~15	46	106
	Congenital abnormality	16 ~ 21, or trisomy 21 and monosomy 16	46	261
	Pseudo-hermaphroditism	21 ~ Y	46	262
	Turner's syndrome	Enlarged X	46	263
	Familial Marfan's syndrome	Enlarged satellite	46	264
	Transmissible hypospadias	Y deletion	46	265
	Gonadal dysgenesis	X or Y deletion	46	266
	Auricular septal defect	2 ~ (6–12)	46	267
	Familial malformation of central nervous system	Enlarged satellite	46	268

TABLE III. LETHAL AND DETRIMENTAL EQUIVALENTS DERIVED FROM STUDIES OF OFFSPRING FROM FIRST-COUSIN MARRIAGES

(*Modified after Newcombe*[209])

		Consanguineous (first cousin only)			*Control*					
Place	*Condition*	*Affected*	*Total*	*Frequency (%)*	*Affected*	*Total*	*Frequency (%)*	*Difference (%)*	*Lethal or detrimental equivalent*	*Reference*
U.S.A.										
	Infant death; juvenile death	637	2,778	22.93	134	837	16.01	6.92 ± 1.50	2.21 ± 0.48	270
	Death under 20 years	113	672	16.82	370	3,184	11.62	5.20 ± 1.55	1.66 ± 0.50	271
	Miscarriage	36	248	14.52	25	194	12.89	1.63 ± 3.29	0.52 ± 1.05	
	Still birth; neonatal death	7	212	3.33	5	196	2.98	0.35 ± 1.73	0.11 ± 0.55	272**
	Infant death; juvenile death	14	205	6.34	1	164	0.61	5.73 ± 1.81	1.83 ± 0.58	
	Abnormality	31	192	6.15	16	163	9.82	6.33 ± 2.91	2.03 ± 0.93	
France										
	Still birth	43	1,043	4.12	84	4,094	2.05	2.07 ± 0.65	0.66 ± 0.21	
	Infant death	87	982	8.86	182	4,010	4.54	4.32 ± 0.96	1.38 ± 0.31	273
	Death from 1 to 30 years	104	886	11.74	227	3,822	5.94	5.80 ± 1.12	1.86 ± 0.36	274
	Abnormality*	169	1,043	16.20	176	4,094	4.30	11.90 ± 1.18	3.81 ± 0.38	
Japan										
	Still birth; neonatal death	125	2,798	4.47	2,091	63,145	3.31	1.16 ± 0.40	0.37 ± 0.13	
	Infant death	54	822	6.57	808	17,331	4.66	1.91 ± 0.88	0.61 ± 0.28	40
	Juvenile death	41	352	11.65	31	567	5.47	6.18 ± 1.96	1.98 ± 0.63	275
	Abnormality*	69	4,845	1.42	651	63,796	1.02	0.40 ± 0.17	0.14 ± 0.05	

* Indicates some overlap with the preceding classes.

** Controls drawn from offspring of sibs of the consanguineous pair.

See also Böök[276] who found no significant difference in the mortality in small samples of offspring of first-cousin and control marriages, but a considerably greater proportion of the cousin offspring having hereditary diseases (16 versus 4 per cent), and having lower than average intelligence (26 versus 15 per cent). Since the individual offspring were observed for varying periods of time the mortality data are not readily presented in the above form. An average of three recessive deleterious genes per person is estimated from these data.

TABLE IV. ESTIMATED MUTATION RATES AT LOCI DETERMINING AUTOSOMAL DOMINANT DISEASES IN MAN

(Modified from Stevenson[277] and Penrose[39])

Trait	*Region*	*Estimated rate/locus/gen. (× 10^{-6})*	*Reference*
Epiloia	England	8	278
Achondroplasia	Denmark	43*	279
	Sweden	68*	280
	Northern Ireland	13	281
Aniridia	Denmark	5	282
	Michigan	4	283
Microphthalmos	Sweden	5	284
Retinoblastoma	England	15	285
	Michigan	23	286
	Northern Ireland	29	287
	Germany, Fed. Rep. of	4**	288
Neurofibromatosis	Michigan	100+	289
Huntington's chorea	Michigan	5	290
Arachnodactyly	Northern Ireland	6	291
Acrocephalosyndactyly	England	3	292

* This estimate probably includes phenocopies.
** This figure is adjusted for presumptive phenocopies.

TABLE V. ESTIMATED MUTATION RATES AT LOCI DETERMINING SEX-LINKED DISEASES IN MAN

(Modified from Stevenson[277])

Trait	*Region*	*Basis of estimation $\mu = 1/3\ (1 - f)\ x$**	*Estimated rate/locus/gen. (× 10^{-6})*	*Reference*
Haemophilia	England	$f = 0.25$ Est $x = 0.8 \times 10^{-4}$	20	293
	Denmark	$f = 0.286$ Est $x = 1.33 \times 10^{-4}$	32	294, 295
	Denmark and Switzerland	$f = 0.333$ $x = 489/4{,}092{,}025$	27	296
Duchenne type muscular dystrophy	Utah, USA	$f = 0$ $x = 18/63{,}000$	95	297
	Northern Ireland	$f = 0$ $x = 48/271{,}896$	59	298
	England	$f = 0$ $x = 16/138{,}403$	39	299
	England	$f = 0$ $x = 15/105{,}310$	47	300
Limb girdle muscular dystrophy	Northern Ireland	**	34	51
Recessive deaf-mutism	Northern Ireland	**	13	51

* μ = Mutation rate/locus/generation.
f = Relative genetic fitness.
x = Frequency of trait in population.
** Estimates made by special methods.[51]

TABLE VI. ESTIMATED MUTATION RATES AT LOCI DETERMINING AUTOSOMAL RECESSIVE DISEASES IN MAN

(Modified from Penrose[39])

Trait	*Region*	*Basis of estimation* ($\mu = (1 - f)\, x^*$)	*Estimated rate/locus/gen.* ($\times 10^{-6}$)	*Reference*
Juvenile amaurotic idiocy	Sweden	$f = 0$ Est $x = 3.8 \times 10^{-5}$	38	301
Albinism	Japan	$f = 0.5$ Est $x = 5.5 \times 10^{-5}$	28	302
Ichthyosis congenita	Japan	$f = 0$ Est $x = 1.1 \times 10^{-5}$	11	302
Total colour blindness	Japan	$f = 0.5$ Est $x = 5.5 \times 10^{-5}$	28	302
Infantile amaurotic idiocy	Japan	$f = 0$ Est $x = 1.1 \times 10^{-5}$	11	302
Amyotonia congenita	Sweden	$f = 0$ $x = 1/44109$	23	280
Epidermolysis bullosa	Sweden	$f = 0$ $x = 2/44109$	45	280
Microcephaly	Japan	$f = 0.02$ Est $x = 5 \times 10^{-5}$	49	303
Phenylketonuria	England	$f = 0$ Est $x = 2.5 \times 10^{-5}$	25	54

* μ = Mutation rate/locus/generation.
f = Relative genetic fitness.
x = Frequency of trait in population.

TABLE VII. STUDIES OF TIME-DISTRIBUTION OF DOSE—MODIFICATION OF PRE-MUTATIONAL DAMAGE AND ASSOCIATED PHENOMENA

Material	*Radiation*	*Mutations*	*Phenomenon*	*Remarks*	*Reference*
Mouse spermatogonia	X, γ	Recessive visibles and lethals at seven selected loci	Fourfold reduction in effect at low dose-rate	Differential viability of cells, radiation quality eliminated	119,124, 127, 132
Mouse oöcytes	X, γ	Recessive visibles and lethals at seven selected loci	More than fourfold reduction at low dose-rate	Inter-cell selection differential viability, radiation quality eliminated	121,123 304
Drosophila oögonia	γ	Sex-linked recessive lethals	Reduced effect at low dose-rate		128
Silkworm, early stages of spermatogonia and oögonia	X, γ	Egg-colour mutants at two specific loci	Reduced effect at low dose-rate	After elimination of cell selection and later stages	97
Dahlbominus, wasp oögonia	γ	Eye-colour mutants in female larvae	No effect at intensity differences of 1,000 r/min and 0.17 r/min	Probably oögonia	130
Drosophila spermatogonia	γ	2nd chromosome recessive lethals	No intensity effect at 2,000 r/min and 2.0 r/min	Total dose 3,000 r	129
Drosophila spermatogonia	γ	2nd chromosome recessive lethals	Reduction at intensity differences from 0.01 r/min to 0.10 r/min	Total dose 200 r	129
Drosophila spermatogonia	X	Sex-linked recessive lethals	Reduced (?) effect of fractionated dose	Shifts in brood pattern of mutation rates cannot be excluded	305
Drosophila spermatogonia	X	Sex-linked recessive lethals in ring-X chromosome	No effect of dose fractionation; enhancement by post-treatment with N_2; reduction by pre-treatment with chloramphenicol	Intensity of radiation for fractionation and N_2 post-treatment 55 r/sec	153,154, 155, 156
Drosophila spermatogonia (?)	X	Sex-linked lethals	Decrease by feeding of larvae with actinomycin D and penicillin	Stage not defined, probably spermatogonia	306, 307

TABLE VII. STUDIES OF TIME-DISTRIBUTION OF DOSE—MODIFICATION OF PRE-MUTATIONAL DAMAGE AND ASSOCIATED PHENOMENA (*continued*)

Material	*Radiation*	*Mutations*	*Phenomenon*	*Remarks*	*Reference*
Drosophila spermatids and spermatocytes	X	Sex-linked recessive lethals in ring-X chromosome	Reduced effect of dose fractionation and of pre-treatment with chloramphenicol and ribonuclease; enhancement by post-treatment with N_2; both increase and decrease by post-treatment with HCN	Gene mutations and possibly small deletions; radiation given at high dose-rates; inhibition of metabolic repair and delay of mutation fixation	152,153, 154,155, 156, 157
Drosophila sperm	X	Sex-linked recessive lethals in ring-X chromosome	Increase by pre-treatment with ribonuclease and chloramphenicol		153, 154
Drosophila sperm	X	Sex-linked recessive lethals; chromosome breaks	Reduced (?) effect of dose-fractionation in absence of O_2	Critical period ~ 40 min; critical dose for breaks	158, 161
Drosophila sperm	X	Chromosome breaks	O_2 affects both breakage and rejoining of chromosome fragments; no saturation of O_2 sensitivity systems	Radiation given in N_2, air, or at 1 At of O_2	163
Drosophila oöcytes	X	Half-translocations, detachment of attached X-chromosomes	O_2 affects both breakage and restitution of breaks	N_2 between X-ray fractions, or as a post-treatment increase half-translocation frequency	308, 309
Habrobracon oöcytes	X	Hatchability of eggs treated in first meiotic metaphase	Post-treatments with N_2 and CO increase radiation damage	Realization of potential radiation damage	310
Drosophila spermatids	X	Translocation	Cyanide post-treatment increases frequency	After both low and high dose-rates; CN delays restitution of breaks, more translocations	152, 311
Paramecium	X, UV, α	Recessive lethals expressed after autogamy	Effect of time between irradiation and chromosome duplication	Effect of various post-treatments (nutrition, metabolic inhibitors)	139, 140
E. coli; *Streptomyces* spores; *Serratia*	UV, X	Biochemical reversions, "EMB colour"	Observe mutation frequency decline, mutation stabilization, mutation fixation, and mutation expression	Pre- and post-treatment with various temperatures, nutritional factors, and metabolic inhibitors, relations to protein, RNA and DNA synthesis	143,144, 145,146, 147,148, 149
Neurospora	UV	Biochemical mutation	Protein synthesis decreases mutation at low UV doses, but increases mutation at high doses	RNA derivatives increase mutation frequency at low doses only	150
Trifolium		Somatic mutations at leaf marking locus	Reducing effect of dose fractionation	Protection by dose of 12.5 r, dependent on O_2-tension and temperature	312
Vicia	X, Neutron	Chromosome breaks	Process of rejoining inhibited by radiation	Repair requires cellular metabolism and protein synthesis	313, 314

TABLE VIII. EFFECT OF IRRADIATION OF MOTHERS ON THE PROPORTION OF MALE OFFSPRING

Country	Control		Dose range rads	Irradiated		Reference
	No. live births	Per cent male		No. live births	Per cent male	
Japan	43,544	52.085	ca. 8	19,610	51.979	227, 315
			ca. 75	3,958	51.440	
			ca. 200	2,268	51.190	
U.S.A.	Control not available		50–200	407	49.1	235
France	355	54.6	200–400	161	44.7	236
	674	50.1	2–10	797	52.2	
Netherlands	225	53.3	300–600	221	48.0	238

TABLE IX. EFFECT OF IRRADIATION OF FATHERS ON THE PROPORTION OF MALE OFFSPRING

Country	Control		Dose range rads	Irradiated		Reference
	No. live births	Per cent male		No. live births	Per cent male	
Japan	43,544	52.085	ca. 8	5,168	51.587	227, 315
			ca. 60	1,226	53.263	
			ca. 200	753	52.722	
	609	51.72	Many doses of unknown amount	4,201	53.64	237
	Average for Japan	51.24				
U.S.A.	3,491	52.42	Many small doses	4,277	51.39	225
France	1,185	51.5	200–400	656	56.1	236
	1,926	52.7	2–20	1,394	46.0	
Netherlands	828	46.6	300–600	635	52.3	238
	657	52.3	1–10	668	53.4	

TABLE X. NATURAL AND INDUCED MUTATION RATES AT SEVEN SPECIFIC LOCI IN ADULT MOUSE SPERMATOGONIA AND OÖCYTES

Source	Details of irradiation		No. of offspring	Mutations in spermatogonia		Reference
	Total Dose (r)	Dose Rate (r/min)		No. of mutations	Mean no. of mutations per locus per gamete ($\times 10^{-5}$)	
X-ray	300	80–90	40,408	25	8.84	119
X-ray	600	80–90	119,326	111	13.29	119
X-ray	1000	80–90	31,815	23	10.33[a]	119
X-ray	600 + 400[b]	80–90	4,904	10	29.13	121
X-ray	600	60–70	10,761	11	14.60	126
Co^{60}	600	24	44,352	33	10.63	121, 316
X-ray	600	9	28,339	14	7.06	317
Cs^{137}	600	0.8	27,840	10	5.13	125
Cs^{137}	300	0.009	58,457	10	2.44	121, 316
Cs^{137}	516	0.009	26,325	5	2.71	121
Cs^{137}	861	0.009	24,281	12	7.06	121
Co^{60}	603[c]	0.007–0.009	10,763	2	2.65	126
Co^{60} and radium	37.5[d]	0.0011–0.0078	63,322	6	1.35	318
Cs^{137}	86	0.001	56,993	6	1.50	121
Control	—	—	544,897	32	0.84	119, 121, 316, 318
				Mutations in oöcytes		
X-ray	400	92–96	12,853	16	17.78	121, 123
Cs^{137}	400	0.8	36,083	13	5.15	304
Co^{60}	600[e]	0.05	10,117	1	1.41	319
Cs^{137}	258	0.009	27,174	2	1.05	121, 123
Control	—	—	98,828	1	0.14	121, 123, 316

[a] For a possible explanation of the low mutation frequency, see paragraph 83 above.
[b] The two fractions were delivered 15 weeks or more apart.
[c] Delivered in 90 12-hr. or 16-hr. days.
[d] Delivered in 5, 25, or 35 16-hr. nights.
[e] Delivered in 12 16-hr. nights.

TABLE XI. POLYGENIC TRAITS: MUTATIONAL DATA

Material and characters	*Treatment*	*Method*	*Results: increase in genetic variance*	*Comments*	*Reference*
Drosophila melanogaster					
Abdominal bristle number.....	(*a*) 1,800 r per generation as adults	Response to selection for high and low lines	3.3×10^{-5} rad Not significant but ≥ 0.006/generation	Natural genetic variance cited as 5 units for abdominals and 1.7 units for sternopleural	320
	(*b*) Nil	(10/25)			
Drosophila melanogaster					
Abdominal bristle number.....	Nil	Analysis of increased variance associated with second chromosome	0.0014/generation		321
Sternopleural bristle number.....	Nil	Ditto	0.0004/generation		
Drosophila melanogaster					
Abdominal bristle number.....	Nil	Analysis of variance associated with second chromosome	0.006/generation		322
Sternopleural bristle number.....	Nil	Ditto	0.002/generation		
Drosophila melanogaster					
Abdominal bristle number.....	?	Regression of variance on dose for large chromosomes	8.7×10^{-5}/rad		
Sternopleural bristle number.....	?	Ditto	3.5×10^{-5}/rad	Details of X-ray treatment and dose not given	
Drosophila melanogaster					
Sternopleural bristle number.....	(*a*) 3,000 r X-rays every generation	Selection for high no. (*a*) Top 15% every generation	$\geq 4.7 \times 10^{-5}$/rad*		323
	(*b*) 3,000 r X-rays every other generation	(*b*) Top 15% every other generation	$\geq 2.5 \times 10^{-5}$/rad*		
Rice					
Heading date.....	6 or 12,000 r X-rays to seeds	Variance analysis 5 generations after irradiation of highly inbred line	1.5×10^{-5}(day)^2/rad	If suppose inbreeding system leaves variance equiv. of 3–5 generations of spontaneous mutation, can calculate spont. rates of $8–10 \times 10^{-3}$(day)^2/generation for heading date and $6–7.5 \times 10^{-2}$(cm)^2/generation for plant height	324
Plant height.....	Ditto	Ditto	8.4×10^{-5}(cm)^2/rad		
Maize					
9 attributes.....	Nil	Analysis of shifts in plot means over 6 generations of selfing doubled monoploids	Average of 4.5×10^{-2} mutations per attribute per gamete	Variance analysis failed to give significant results because of high environmental component	325
Arabidopsis thaliana					
Logarithm of flowering data.....	0–150 kr X-rays to dry seeds		2×10^{-5}/rad	Variance of flowering data in natural populations not known. Controls probably not significantly different from ~ 0.00043; good linearity with dose obtained	326

* Secretariat calculation.

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back to first page