REPORT OF THE UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECTS OF ATOMIC RADIATION

GENERAL ASSEMBLY
OFFICIAL RECORDS : SEVENTEENTH SESSION
SUPPLEMENT No. 16 (A/5216)

UNITED NATIONS
New York, 1962

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 B

FUNDAMENTAL RADIO-BIOLOGY

CONTENTS

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.

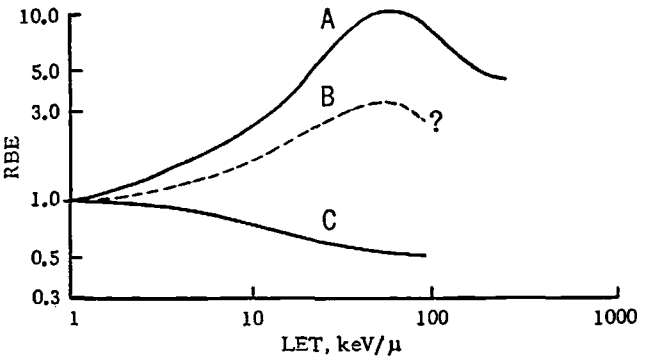

Figure 1. Variation in RBE with LET, for biological materials irradiated in aerobic conditions[43]
A: plant cells[577–582]
B: animal cells[203, 577, 583–585]
C: two strains of bacteria[183, 586–587]

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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