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CHROMOSOME ABERRATIONS PRODUCED BY NEUTRONS

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Walter N. Hittelman

(M.S. Thesis)

August 1968

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Walter N. Hittelman

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ABSTRACT

This paper discusses the formation of aberrations in the chromosomes of plant and animal cells following irradiation by neutrons. While the neutron is the focus of attention, a discussion of its effects is embedded in a general study of chromosome aberrations produced by all types of radiation, including gamma-, alpha-, and X-rays, protons and electrons.

The paper is the result of a literature search. It examines the following topics: chromosome structure, causes of "breaks", chromosome repair, formation of aberrations, linear energy transfer and relative biological effect considerations, aberration kinetics, and oxygen considerations.

INTRODUCTION

The following paper is a discussion of the production of chromosome aberrations by all forms of radiation, neutrons in particular. It is the result of an extensive literature search and it brings together important ideas in the field of aberration production. It is of interest to the researcher as it confines all the literature and experimental work into a few pages. It presents many problems needing further study and gives enough of a background to seriously consider experimental work in this area. The ideas presented herein can also be employed in areas outside of chromosome aberration production. For example, studies involving cell aging and cell death are very closely related to chromosome aberration studies. The discussion of aberrations also sheds much light on chromosome structure during mitosis and meiosis, DNA synthesis, radiation biochemistry, chromosome repair, just to mention a few topics.

The paper is divided into eight sections, each dealing with a particular aspect of the total problem. The first section is concerned with a discussion of chromosome structure. It shows that while it is difficult or nearly impossible to determine the structure of the chromosome completely with present techniques such as the light and electron microscopes, there is enough evidence to warrant the postulation of two general models. One idea suggests that the chromosome is composed of a single strand or single strands linked together end-to-end. This single nucleohistone strand is then looped, folded, and coiled to form the observed chromosome. The alternative model suggests that the

chromosome is composed of many of these strands running along side one another. This second model also allows for loops, folds, and coils. This section cites evidence for both these ideas and discusses the implications of both.

The second section discusses the biochemical aspects of radiation damage to chromosomes, indirect and direct. The former effect is a result of the diffusing radiolysis products of water, and the latter effect is due to ionizations within or very near to the chromosome itself. While this section shows that the chromosome can be attacked at any structural level, the bases of the nucleotides seem to be the most sensitive to radiation damage.

The third section is concerned with chromosome repair. Cells can sometimes repair chromosome damage, although it is not known how long this takes nor the mechanism involved. It is shown how the repair ability varies with the dose and type of radiation involved, as well as the stage of the cell cycle.

The fourth section discusses the actual mechanisms of aberration formation, after radiation damage to the chromosome. Two general models for the mechanism are presented and compared: the breakage-and-exchange model and the exchange model. A comparison of the aberration type with the stage of the cell cycle is considered, as well as a discussion of gaps in chromosomes.

The fifth section is concerned with the linear energy transfer (LET) of the particular radiation type and how this factor may influence the type and frequency of aberrations. The LET, or the number of energy loss

events per unit length of the ionizing particle track, is found to be a useful indication of radiation quality.

The sixth section discusses the relative biological efficiency (RBE) of the various radiation types. The RBE provides a method for comparing the amount of damage inflicted on the cell or chromosome by different forms of radiations. It is shown that the RBE is dependent on the LET and dose of the radiation particle, as well as on the stage in the cell cycle, the type of tissue involved, and the chromosome volume in the cell.

The seventh section is concerned with aberration kinetics. Two general mathematical formulations for the rate of aberration formation are presented and compared. One model involves the determination of the aberration yield only as a function of dose. The second method involves a statistical derivation of aberration yield as a function of the characteristics of the chromosome and of the particular radiation type.

The eighth and last section is concerned with the effects of oxygen conditions during experimentation. It is shown that the amount of effect produced on the aberration yield by the oxygen environment is dependent upon the form of radiation involved. The effect of oxygen conditions is also quantitatively derived.

It is hoped that the reader recognizes the logic of the topic order. Before one can discuss the mode of aberration production, for example, one must understand the structure of the chromosome as well as how the chromosome can be both damaged and repaired. Once the problem is well defined in this manner, the comparative and quantitative aspects can be better appreciated.

I. CHROMOSOME STRUCTURE

One of the problems involved with the study of chromosome aberrations is that investigators still do not know the structure of the chromosomes themselves in great detail. This first section of the paper discusses the problem of chromosome structure. Several models have been proposed within the last several years, but, as will be shown, none of the models adequately account for all the experimental observations that have been obtained. Thus some models fit some results, other models fit other results, but the correlation of experiment and theory is far from complete. As a result, the ultimate shape and dimensions of chromonemata,[1] or in other words, the packing pattern of the nucleoprotein in the chromosomes, is still relatively unknown.

The difficulties involved in defining the structure of chromosomes is considered first. This includes a discussion of practical experimental problems as well as the problem of blending experimental and theoretical considerations. Two models for chromosome structure are introduced. These include the single strand and the multistrand hypotheses. The packing of the chromosomes is included in the discussion of the two models.

The resolution of the structure of chromosomes is not a trivial problem, especially with the techniques now available to the investigator. Studies are difficult because the structural details are generally below the level of resolution of the light microscope yet above the

size level at which the thin sections required for resolution in electron microscopy can give a complete picture.[2] Also, the complexity of the structure of the chromosome has lessened its amenability to electron microscope studies. The problem is straightforward. Since the chromosome is thought to consist of a very complex structure that is built of coils upon coils, any section cutting across the superstructure will show only a granular appearance.[3] Thus the sorting out of the structure components is difficult. For example, Bloom and Leider describe the components of chromosomes from cells, fixed in neutral formalin in Tyrodes's solution and stained with phosphotungstic acid and viewed with the electron microscope, in the following manner:

> . . . parts of the chromosome consist of (a) a homogeneous or finely fibrillar material (component A) filling the meshes of (b) an irregular network with bars 40 to 300 A in diameter, some of which continue into a similar inter-chromosomal network. DNA-steretic portions of the chromosomes (the parts affected by radiation) consist mainly of this network and only small amounts of component A, which presumably contains the DNA.[4]

Other difficulties arise from the nature of the chromosomes themselves. For one, the tertiary[5] or three-dimensional structure in the nucleoprotein components of chromosomes has been found to be highly unstable. In addition, the chromosomes present a changing structure relative to the cell division cycle. Even during interphase, when some of the most important and significant changes are occurring, the chromosomes present little structure amenable to analysis with either the light

or electron microscope.[6]

There are also theoretical problems associated with the resolution of the structure of the chromosome. For example, in the resting nucleus, where the chromatin[7] is not condensed, structure detected at the 100-A level must encompass several tenets of genetics which include informational linearity and the mechanism for transferring information into m-RNA. In addition, one must expect eventually to see the orderly evidence of the process of precise, semi-conservative self-duplication of DNA occurring at this stage.[8] The structure must also account for the fact that at this time the chromosomes change from acting as single units in the formation of aberrations to the state in which they react as two units.[9]

To this date, most of the conceptual pictures presented by investigators have been encompassed by three possible arrangements of the chromosome. These include the following: (1) one long continuous DNA double helix with its associated protein folded and coiled to form the visible chromonema[10] of a typical chromosome; (2) a regular arrangement of molecules of DNA or nucleoprotein particles linked together in tandem with non-DNA linkers, or, alternatively, attached by their ends to a shorter central axis; and (3) a multistranded complex of DNA, protein, and perhaps other constituents with many DNA double helices forming the axis of the chromonema.[11]

Recent investigation has been guided by the hope for a simple structure. The chromosomes of bacteria and viruses have been found to be composed of a single nucleohistone strand or linear segments of

nucleohistone joined by segments of protein into a single strand. Genetic considerations based on the overwhelming evidence for linear genetic information residing in a linear DNA molecule has lead to the hope that the chromosomes of higher plants and animals might be structured similarly. If this were true, the chromatid[12] of classical cytogenetics, which arises by self-duplication of the chromosome, would also be single stranded. All complexity seen in electron micrographs above the 40-A of possibly 100-A level would be the result of coiling or packing of the single strand.[13]

The folded, coiled and looped model of the chromosome has been interpreted by some to occur in some lower organisms. The lampbrush chromosomes of Triturus Viridescens[14] in the diplotene stage of meiosis[15] was found to be paired although joined by chiasmata[16] at a few points. Each chromosome is thought to possess an axis along which chromomeres[17] are located. Paired loops project from the chromomeres. Stretching experiments have found the lampbrush chromosome to be composed of two continuous strands. The dimensions of these strands are found to be relatively enormous. In fact, the length of the intact structure may reach 1 mm and that of a fully extended chromatid approaches 5 cm. In 1958, Gall reported that the chromomeric axis of the lampbrush chromosome has a diameter of 200 to 400 A in electron micrographs. This suggests that the chromatid has a diameter of 150 to 200 A.[18] Similarly, in the oocyte of amphibians, a very much elongated chromosome is thought to exist. This chromosome then reverts to a regular mitotic type at the following division. Oocyte chromosomes are found to be 500 to 800

microns long in diplotene and 15 to 20 microns in length at later stages. Along the axis numerous loops are observed which are retracted into the main body of the chromosome. When extended, these loops would give the chromosome a contour length of several centimeters.[19] However, the study of chromosome structure does not end with these results. While these experiments indicate the existence of single-stranded chromosomes in these two instances, it does not mean that all chromosomes are single-stranded.

Most investigators envision a doubleness nature in the strand or chromatid. This doubleness idea follows from the fact that the chromatid must function as two subunits during replication at all levels of organization and stage of the cell cycle. There is considerable experimental evidence for this doubleness nature. In favorable preparations of anaphase chromosomes, the rodlike structures have been interpreted to be helically coiled chromonemata. Usually they appear to have a single chromonema. In some fixed preparations, however, the chromatids appear double. In these preparations there may be two helically coiled chromonemata (half-chromatids) which are usually very poorly separated as if the coils were interlocked. Perhaps the axes were otherwise bonded together. These results may be the result of treatment with acid fixative. Fixation in hot water, however, also reveals the doubleness; but <u>in vivo</u> a doubleness has never been demonstrated. In the living state, anaphase chromatids appear to be solid cylinders.[20] Around 1959, Ris showed that two 40-A strands compose the appropriately extracted preparations of isolated calf thymus chromatin. Ris used

saline-versene in his treatment on the chromatin in order to remove the nonhistone protein. Likewise, Zubay and Doty found subunits of 30 A in strands of purified calf thymus nucleohistone.[21]

There has been a considerable amount of experimental evidence for half chromatids. The evidence so far has fallen into five catagories consisting of (1) the types of aberrations induced in late prophase or metaphase by radiation, (2) the types of aberrations induced by radiation at the end of G_1[22] before the chromosomes have replicated, (3) experiments in which the chromosome structure has been unraveled by treatment with enzymes or other agents, (4) the distribution of labeled DNA among the chromosomes at mitosis subsequent to labeling, and (5) experiments in which the effects of incorporated isotopes were expressed in succeeding generations.[23] For example, there is evidence that the chromatid functions as two subunits. This occurs in nonreciprocal recombination[24] and in the induction and segregation of mutants induced by base analogs.[25] However, in chemically and radiation-induced breakage and exchange as well as in reciprocal recombination and sister chromatid exchanges the chromatid acts as a single unit except during prophase. On the other hand, the pattern and frequency of sister chromatid exchanges, as will be seen later in the paper, indicate that each chromatid is composed of two unlike subunits. This occurs even though the exchanges are only between whole chromatids.[26]

The discussion now turns to the question of whether the chromosome is single or multi-stranded. In interphase nuclei, fibrils about 100 A in diameter are seen in cells of many species. Often these 100-A fibrils

are paired, indicating a multi-stranded structure. Nevertheless, the 100-A fibrils which appear in spermatids before the chromosomes have been reproduced may still contain two DNA double helices since these fibrils have been reported to change to 40-A fibrils in most sperm nuclei. According to the appropriate model, this change in diameter has been postulated to come about in several ways. One idea suggests the decrease in diameter results from a multi-stranded chromosome coming apart. Another idea suggests that the diameter change results from a change in the protein with consequent variations in the pitch of a super helix of the DNA double helix.[27]

The single-strand theory asserts that the unit structure of a chromosome is a strand 100 A in diameter, composed of two fibrils 35 to 40 A in diameter. These strands are thought to consist of nucleohistone. Relatively enormous lengths of such strands must be packed into the volume of a nucleus or metaphase chromosome of ordinary size. Such packing might require the folding, looping, and/or coiling at size levels varying from tens of angstrom units to a few microns.

The coiling of the chromosome is thought to be a function of a molecular level relationship between the DNA and the histone and perhaps other proteins or even lipids. One investigator has speculated that histone may not only be wrapped around DNA but may also form cross links between the gyres of DNA coiled at the next level above the Watson-Crick helix.[28] Cations are thought to have an important function in the coiling of chromosomes. Somers has shown that metaphase chromosome of cultured Chinese hamster cells may be uncoiled by removing Mg

and Ca from the suspending medium. The importance of Ca has been confirmed by Chorazy and associates for the maintainance of structure of mammalian metaphase chromosomes which are isolated and unfixed. In addition, Chorazy et al. report that urea and deoxycholate will disperse condensed chromosomes. The enzymes trypsin or DNAase also cause rapid (10 to 20 minutes) and complete disintegration. These experiments suggest that condensed chromosomes owe their structure almost exclusively to a complex of DNA and basic protein. The cations would then affect the tertiary structure of nucleohistones.[29]

The degree of coiling is said to change throughout the cell cycle. At the end of anaphase, there may be a further condensation of the chromosomes from the metaphase stage. Following this phase, the chromosomes expand somewhat in telophase and chromonemata become less tightly coiled. The chromosomes then appear to fuse and nearly fill the reformed interphase nucleus. As the nuclei grow, the chromosomes may not continue to fill the nucleus. This is particularly true in cells that are not to divide again. Their disposition in most interphase nuclei is difficult to follow either by the use of the light microscope or the electron microscope.[30]

The coiled chromosome model does not, however, explain some evidence concerning the diameter and length of the chromosome when extended. With regard to diameter, from a study of structures such as prophase and anaphase chromosomes, the chromonema is found to be on the order of 0.1 to 0.3 microns. This is several orders of magnitude larger than the extended nucleohistone fiber diameter of 30 A.[31] With regard to length,

the DNA of phage T4 is more than 50 microns long when fully extended. The chromosome of E. Coli is thought to be perhaps 50 times this length (more than 2.5 mm). Similarly, some of the largest chromosomes might be more than a meter in length. Even if these chromosomes were composed of several strands of DNA, they would still be several centimeters in length.[32] Two additional models attempt to account for the seemingly large diameter and length. These models postulate loops as a part of the chromatid axis and linkers connecting DNA chains together.

In lampbrush chromosomes, the DNA strand is believed to run continuously along the chromonema, take part in some complex coiling in the chromomere, extend out into each side loop and return to join the chromomere before continuing in the chromonema.[33] The evidence for these loops is as follows. A few years ago, Callan and MacGregor showed that DNAse could rapidly sever the loops of the isolated lampbrush chromosomes.[34] Although some cytologists had maintained that loops were part of the chromatid axis, this was the first demonstration that the DNA was continuous through the loops. In addition to the DNA in the loops, the chromomeres along the axis were found to be Fuelgen positive and therefore to contain much of the DNA.[35]

In this model, the chromomeres consist of two closely associated chromatids. While it is found that a great amount of RNA and protein is attached to the loops, neither RNAse nor proteases will sever the loops. When this protein and RNA coating is dissolved with concentrated KCl solution, a fine fibril is reported to be revealed. This fine fibril is presumably DNA. Electron micrographs of this fibril reveal its diameter

to be sometimes as small as 40 to 50 A. In studies on the rates at which the loops of the Lampbrush chromosomes are broken by DNAse, Gall (1962) has been able to obtain evidence that the structural axis is composed of a single DNA double helix. However, other researchers have done enzyme kinetic experiments on the loops and have found that the loops exhibit multi-stranded kinetics. Nevertheless, if Gall's work is taken as correct, two ideas have been evidenced: the loops are part of the axis of single chromatids, and the loops are found to be perhaps composed of a single fibril or more. The joining of these ideas suggest that these elongated structures have a single DNA double helix as their linear component. DNAse also produced breaks between the chromomeres along the axis of the paired chromatids. The breakage follows the kinetics in some experiments for structure held together by a pair of DNA double helices.[36] However, here again, more recent experiments have indicated kinetics corresponding to a multi-stranded structure. In this discussion, it should be remembered that this evidence for a single-stranded structure holds only so far for the Lampbrush chromosomes and not necessarily for other chromosomes.

Another useful model in the single-strand hypothesis is to visualize the employment of linkers in the folding and unwinding of a long piece of DNA. The essential feature of this model is a DNA double helix with a regular sequence of linkers alternating in the two unfolding chains. These linkers are located opposite a gap in the complementary chain.[37] The evidence for this hypothesis comes from several sources. In 1960, Anderson and Fisher studied viscosity changes in rat-thymus nuclei

suspended in 1M NaCl induced by DNAse, changes in pH and X-rays. Their results agree with the idea that DNA molecules are strongly linked end-to-end by protein and more weakly crosslinked laterally by protein. In 1960, Dounce and Sarkar also concluded that DNA molecules are held together end-to-end by covalently linked nonhistone protein. In 1963, on the basis of x-ray diffraction patterns and electron micrographs, Zubay proposed that oriented nucleohistone gels are composed of longitudinal DNA molecules with lateral histone bridges lying at 60 degrees to the DNA molecules in the large groove. He extended this model to chromatin by suggesting that supercoils of DNA are stabilized laterally by similarly oriented histone bridges.[38]

More specifically, the model suggests several types of linkages. The first type of linker needed is one to join 5' OH or 5' phosphate groups at the ends of two polynucleotide chains. This results in a reversal in polarity. This is called a 5' linker. Their complementary chains are assumed to be joined by a 3' linker, that is, at the 3' OH group. The chromatid is then assumed to consist of a series of tandemly linked segments (replication units) of DNA with a 3' OH group linked to a 3' OH group and a 5' end linked to another 5' end at each operator site.[39] The 5' linker is thought to be formed by covalent bonds. These would be as stable as the phosphodiester linkages[40] along the polynucleotide chains. The regular 3' linkers are thought to consist of two phosphoserine residues[41] coupled to the terminal nucleoside of DNA chains by an ester linkage. This is the same way that amino acids are coupled to transfer RNA. A diphosphate bridge could then couple two chains with

a reversal in polarity. This hypothesis is supported by evidence that sperm DNA contains about one phosphoserine residue per thousand nucleotides.[42]

A third type of linker is called an H linker. According to this model, its function is to stabilize the chromatid by holding it in a folded ladder-like arrangement. The H linker is thought to be a type of polymer joining alternative 5' linkers along two axes. These would represent the half-chromatids. During the following replication, these would form part of the linear axis of the two sister chromatids. A new set would be formed on each chromatid at prophase when it became double, i.e., when half-chromatid bridges can be induced. In rapidly dividing cells these linkers might be closed most of the time. In cells where the chromosome acts as a single unit in breakage and reunion, these linkers would have to be assumed to be open or rather labile. Since protein synthesis appears to play a role in the reunion of chromosomes, the H linkers are assumed to consist of polypeptides.[43] However, stability tests and kinetics of shear breakage seem to indicate no preferential weak points along the molecule. Thus, one might view T2 and T4 phage DNA as a single linear duplex and postulate the existence of special nonnucleotide residues which unite distinct polynucleotide chains. Yet one must realize that if nonnucleotide linkages exist, they must be at least as strong as the internucleotide linkages themselves.[44]

A remaining problem is that of putting the coiling and looping ideas together with the linkage idea. If these models are to both apply, then the loops should open up when the protein or polypeptides are removed. However, proteases and RNAse do not break up the loops. Thus

long stretches of peptides or RNA which might serve as linking material appear unlikely.[45] However, this type of experiment would certainly depend upon the stage in the cell cycle.

While all the evidence and models presented thus far support the proposition that the structure of the chromosome is single-stranded, there is still much debate on the subject. Wolff claims that the preponderance of the cytological evidence indicates that most chromosomes are not single-stranded. For example, recent studies using a technique whereby chromosomes are dispersed on the clean air-water interphase of a Langmuir trough show that chromosomes consist sometimes of fibers with diameters in the 200 to 250 A range as well as in the 500 A range. Moreover, photographs of thin-sectioned, isolated and shadowed chromosomes were interpreted to indicate that a single chromosome consists of many parallel strands. The multiplicity of threads combined with the series of sizes ranging from 40 A to 500 A suggested to Kaufmann and De in 1956 that the early prophase chromosomes of Tradescantia are composed of "as many as 64 identifiable subsidiary strands, assumedly arranged as intertwined pairs to form a hierarchy of pairs of pairs."[46] This hypothesis is tagged the rope hypothesis.

Wolff claims that most of the evidence obtained by light microscopy and by both observational and experimental evidence have indicated that chromosomes can be multi-stranded structures. Furthermore, a comparison of the amount of DNA in closely related species with the genome of these species has indicated that changes in polyteny[47] might very well have occurred. Also, in view of the recent studies on Chironomus indicating

redundancy of genetic information at certain loci, it seems that a multi-stranded structure is very possible.[48] As Wolff puts it, ". . . it now seems that models of single-stranded chromosomes suffer from the same conceptual difficulties that multi-stranded models do."

In order to complete the understanding of the structure of the chromosome, room must be made for the postulation of protein synthesis. There is good evidence that RNA is concentrated at certain areas of the chromosome. In Lampbrush chromosomes, it has been found that RNA exists in some of the side loops. In giant chromosomes, RNA is particularly associated with structures called Balbiani rings of puffs which appear to be expanded chromosomes. These are thought to represent functional areas of the chromosome.[49] Experiments by Huang and Bonner showed that certain histone fractions, when carefully complexed with DNA, suppress the capacity of DNA to support RNA synthesis in in vivo systems while native nucleohistone does support RNA synthesis to some extent. Extrapolation of these findings to chromosomes suggests that a chromosome region which is supporting RNA synthesis may not be occupied by a histone. Such might be the case in an intensely active RNA synthetic system such as the Lampbrush chromosome. Izawa, Allfrey and Mirsky in 1963 showed that the loops of isolated Lampbrush chromosomes can be made to retract in the presence of substances (actinomycin D and certain histone fractions) which are known to complex with DNA and to suppress the synthesis of RNA.[50] Chromosomes are also known to contain large amounts of acidic proteins, sometimes called chromosomin. The spatial arrangement and functional significance of these is at

present relatively unknown, although they may be present in amounts equivalent to histones.[51]

In summary, the elementary chromosome fibril of 100 A probably consists of two 30 to 40 A DNA-histone molecules arranged side by side and possibly cross-linked through histones or other proteins, and possibly linked end-to-end in some species. These fibrils may be replicated many times in each chromatid. The individual fibrils are probably randomly coiled, the whole mass of the chromatid coiled again and the whole structure coiled once more on top of this. Some structures may resemble the Lampbrush chromosomes where it is thought that the DNA strand runs continuously along the chromonema, takes part in some complex coiling and returns to join the chromomere before continuing in the chromonema. The basic proteins, usually histones, seem to be associated with the nucleic acids throughout the entire length of the chromosome.[52]

A model of this sort should be taken into consideration in the latter sections of this paper, especially in the sections on the type of "breaks" and the kinetics of chromosome damage. One of the problems with much of the mathematical theory is that it assumes a single-stranded model rather than a multi-stranded model for the structure of the chromosome. While these types of theories may lead to a cleaner description and better curves, they may also lead aberration studies down the wrong path.

FOOTNOTES FOR SECTION I

1. The chromonemata are the fundamental elements of the chromosomes which are observed in the light microscope as threads.

2. J. H. Taylor, "The Replication and Organization of DNA in Chromosomes," in Molecular Genetics, Part I, J. H. Taylor, Ed. (Academic Press, New York, 1963), p. 68.

3. S. Wolff, International Review of Cytology, in press.

4. W. Bloom and R. J. Leider, Journal of Cell Biology 13, 2, 269 (1962).

5. In order to define a complicated macromolecule in descriptive terms, four basic structural levels have been assigned. Usually proteins are described in these terms. The primary structure is the sequence of the units which make up the chain, such as the amino acids in proteins. The secondary structure refers to the regular configurations of the backbone, such as the right-handed alpha-helical nature of a protein. The tertiary structure is the three-dimensional structure of the macromolecule and describes the extensive coiling or folding compressing the chain into a globular form. The quaternary structure defines the degree of polymerization of the macromolecule unit.

6. J. H. Taylor, op. cit., p. 67.

7. The chromatin is the granular protoplasmic substance in the nucleus of the cell that readily takes a deep stain. The chromosomes are included in the chromatin.

8. B. B. Hyde, "Ultrastructure in Chromatin," in Progress in Biophysics and Molecular Biology, J. A. V. Butler and H. E. Huxley,

Eds. (Pergamon Press, 1965), Vol. 15, p. 131.

9. J. H. Taylor, op. cit., p. 92.

10. Chromonema is singular for chromonemata. See footnote 1.

11. J. H. Taylor, op. cit., pp. 66-77.

12. The chromatids are the two daughter strands of a duplicated chromosome which are still joined by a single centromere.

13. B. B. Hyde, op. cit., p. 134.

14. Triturus Viridescens is one form of a newt. The term "lampbrush chromosomes" is descriptive of the numerous looplike extensions which come off from the central core of the chromosomal filament.

15. Prophase I of the first meiotic division in cells is divided up into several stages. The diplotene stage is the stage in which the chiasmata or the cross-shaped figures representing the exchange of chromosomal material first becomes evident.

16. See note 15.

17. The chromomeres are bead-like areas of increased density along the chromonemata.

18. B. B. Hyde, loc. cit.

19. J. H. Taylor, op. cit., p. 69.

20. Ibid., p. 67.

21. B. B. Hyde, op. cit., p. 132.

22. The interphase stage of the mitotic cycle, or the stage in which the nucleus outwardly seems to be "resting," is divided into three sections, gap 1, S for DNA synthesis, and gap 2. G 1 stands for gap 1, where there seems to be little activity in the nucleus.

23. S. Wolff, op. cit., p. 6.

24. Recombinations are new combinations of linked genes.

25. Base analogs are purines and pyrimidines which differ slightly in structure from the normal nitrogenous bases. Some analogs may be incorporated into nucleic acids in place of the normal constituent.

26. J. H. Taylor, op. cit., p. 97.

27. Ibid.

28. B. B. Hyde, op.cit., pp. 131-137.

29. Ibid., p. 138.

30. J. H. Taylor, op. cit., p. 68.

31. Ibid., p. 69.

32. Ibid., p. 97.

33. John Paul, Cell Biology: A Current Summary (Stanford University Press, 1966), p. 112.

34. Callan and Macgregor, Nature 181, 1479 (1958).

35. J. H. Taylor, op. cit., p. 69.

36. Ibid.

37. Ibid., p. 98.

38. B. B. Hyde, op. cit., p. 141.

39. J. H. Taylor, loc. cit.

40. A phosphodiester linkage is one where two nucleosides are joined together by a phosphate in an ester-type linkage.

41. A phosphoserine residue is a serine molecule attached to the nucleotide through a phosphage linkage.

42. J. H. Taylor, op. cit., p. 108.

43. Ibid., p. 101.

44. Charles A. Thomas, Jr., "The Organization of DNA in Bacteriophage and Bacteria," in Molecular Genetics, Part I, J. H. Taylor, Ed. (Academic Press, New York, 1963), p. 128.

45. J. H. Taylor, op.cit., p. 71.

46. B. B. Hyde, op. cit., p. 133.

47. Polyteny is the state of having many units of many reduplicated chromonemata in close longitudinal association.

48. Sheldon Wolff, loc.cit.

49. John Paul, loc. cit.

50. B. B. Hyde, op. cit., p. 136.

51. John Paul, loc. cit.

52. Ibid.

II. RADIATION DAMAGE TO CHROMOSOMES

One of the problems in the study of chromosomal aberrations produced by neutrons and other forms of radiation is that the biochemical details of interaction are not known in detail. Although some chemical hypotheses have been presented in the literature, many of the ideas regarding the causes of aberrations are based solely upon the agreement of experimental curves with mathematically derived curves. This assumes a particular type of interaction between radiation and the chromosome. Some of these models will be presented later in this section. The problem is compounded when it is realized that the structure of the chromosome is itself much in doubt, as has been seen in the first section of this paper. Nevertheless, in an effort to provide a clue to the mode of aberration formation resulting from irradiation, the subject has been divided into two categories, the direct and indirect effects of ionizing radiation.

Ionizing radiation is thought to produce damage in DNA by a direct effect, produced by ionizations occurring within the molecule. Damage is also produced by indirect action resulting from an attack on the chromosome by diffusing radiolysis products of water. It should be noted that not all investigators believe that the primary damage to the chromosome is the formation of a break. Some investigators feel that the radiation creates a weak spot in the chromosome where, at some time later, aberrations may be formed. In any case, for the sake of convenience, in this paper the damage to the chromosome will be called a break whether or not a break actually exists. After irradiation in

dilute aqueous solutions, damage is thought to be primarily produced by indirect effects. At the high concentrations of DNA within the cell, direct and indirect effects are believed to be of approximately equal importance.[1] This section will first discuss the indirect effects of radiation on the chromosome and then the direct effects. In each case, the discussion will focus on damage to the various levels of structure, ranging from the nucleoprotein level to the individual sugar, base, or phosphate level.

It is important to realize before continuing, however, that radiation is not the only aberration-inducing agent. R. Haynes has pointed out that both ionizing radiation and ultraviolet light, as well as other mutagenic compounds such as the alkylating agents, possess the common property of producing chemical and physical changes in the DNA. To a large extent, the damage produced by these different agents is additive in terms of biological effects.[2]

Aberration formation experiments are performed both in vivo and in vitro. This is important to note since distinctions are not always made between the two experimental conditions. In order to smooth over this difficulty, Gooch has shown with in vivo and in vitro studies with the Chinese hamster, with the South American spider monkey, and with man that radiation-induced chromosome breakage rates are virtually the same for different mammalian species, as well as for different tissues within the same species. His experiments has led him to the belief that in vitro experiments provide accurate estimates of radiation-induced aberration rates in human cells in vivo. Information from tests on

accidentally irradiated men has further substantiated this finding.[3]

As stated before, the indirect effect of radiation on chromosomal aberrations is thought to be due to the diffusing radiolysis products of water. The radiolysis reaction is thought to proceed as follows:

$$H_2O \rightarrow H_2O^+ + e^-$$

$$H_2O^+ \rightarrow H^+ + OH\cdot$$

$$e^- + H_2O \rightarrow H_2O^- \rightarrow OH^- + H\cdot$$

$$2OH\cdot \rightarrow H_2O_2$$

$$H\cdot + H. \rightarrow H_2 .$$

In 1948, J. Weiss proposed that the irradiation of water resulted in the production of H and OH radicals as shown above.[4] In the presence of air, H radicals interact with oxygen giving rise to HO_2 and H_2O_2. Hydrogen atoms are also thought to be formed by the process:

$$H_2O^- + H_3O \rightarrow H + 2H_2O .$$

Some evidence indicates that small quantities of hydrogen atoms may be directly produced from water. This is possibly a result of the dissociation of excited water molecules in the following manner:

$$H_2O^* \rightarrow H + OH .$$

These hydrogen atoms can then dehydrogenate, creating molecular hydrogen by the following general reaction:

$$RH + H \rightarrow R^{\cdot} + H_2 \ .$$

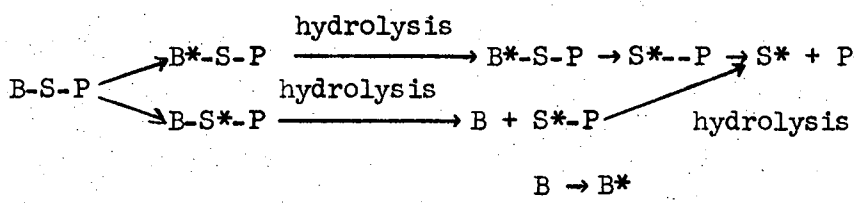
At the same time, H_2O^- can react, as will be seen later, with the unsaturated bonds of the purines and pyrimidines of the DNA or with carbonyl compounds produced during the radiolysis.[5] The yields of the radical products (H, OH) to molecular products (H_2, H_2O_2) has been found to depend on the linear energy transfer (LET) of the radiation used, decreasing with increasing LET. The molecular products (H_2, H_2O_2) arise from recombination of the free radicals (or their precursors) as they diffuse from the tracks of the ionizing particles; hence the dependence on LET.[6]

Around the year 1948, a correlation was found between hydrogen peroxide formation and the production of chromosomal aberrations. This very close parallel between hydrogen peroxide formation in irradiated water and the production of chromosome aberrations led to the suggestion by Thoday and Read that H_2O_2 was a prime factor responsible for the production of aberrations.[7]

The extent to which DNA is damaged by radiation <u>in vivo</u> and <u>in vitro</u> can be profoundly effected by the absence of oxygen, as well as by the presence of sensitizing or protecting chemicals.[8] The existence of a so-called oxygen effect, the experimental and mathematical details of which will be discussed later in the paper, is further substantiated by the fact that the yield of hydrogen peroxide in X-irradiated water is known to be dependent on the presence of oxygen. However, this effect plays a smaller role in the formation of aberrations by neutrons and alpha particles since, in these cases, H_2O_2 is produced independently

of the presence of oxygen. That is, with a densely ionizing radiation the close spacing of OH radicals favors their interaction to give H_2O_2 whether free oxygen is available or not.[9]

Since it is known that the passage of ionizing radiation through the cell induces radical formation, the next step is to understand the effects of the newly created radicals. Several studies have attempted to observe the sites of radical action on the chromosome. Scholes has outlined a generalized schematic representation of the possible modes of radiation-induced damage to nucleotides in aqueous systems. This scheme describes one mode of aberration formation as well. In the following diagram, B, S, P, B*, and S* represent base, sugar, phosphate, chemically changed base, and chemically changed sugar respectively.[10]



The bases of the DNA are perhaps the main targets for radical action in the chromosome. Chemical studies of the changes produced in pyrimidines have shown that at neutral pH in the presence of oxygen, the 4-5 double bond is the main site of attack. The primary product is hydroxy hydroperoxide. The 4-5 bond in pyrimidines has an ethylenic character and it is for this reason that it is attacked. Attack by the radiation-produced radicals also leads to a loss of the chromophoric character[11] of the pyrimidine ring. Furthermore, since the number of double bonds destroyed (as measured by reaction with bromine) corresponds to the number of molecules destroyed, saturation of the 4-5 double bond is thought to

be the only process taking place.[12] Scholes offers the following scheme for the over-all initial processes occurring in irradiated pyrimidine solutions:

$$Py + OH \rightarrow Py(OH)^{\cdot}$$

$$Py(OH)^{\cdot} + O_2 \rightarrow Py(OH)O_2^{\cdot}$$

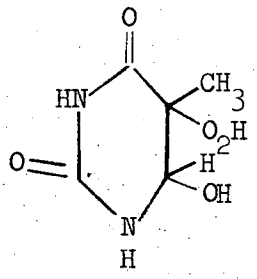
$$O_2 + H \rightarrow HO_2 \rightleftharpoons O_2^- + H^+$$

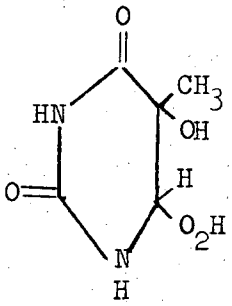
$$Py(OH)O_2^{\cdot} + O_2^- \rightarrow Py(OH)O_2H \quad \text{(Hydroxy hydroperoxide)}$$

$$2Py(OH)O_2^{\cdot} \rightarrow \text{products} + H_2O_2$$

$$2HO_2 \rightarrow H_2O_2 + O_2 \text{ (acid conditions)} .$$

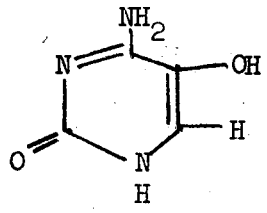
The products of the reactions are unspecified.[13] In the case of thymine, the proposed structure of the hydroperoxide is given by either of the following two structures:[14]



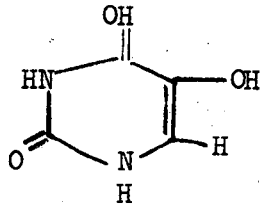


The hydroperoxide of thymine is more stable than that of cytosine. At pH 7 the cytosine derivative is rapidly converted to the glycol. The glycol then gives 5-hydroxycytosine by loss of water or uracil glycol by deamination. In fact, in neutral aqueous media, the stability of the

pyrimidine hydroperoxides follows this order: Thymine h > uracil h > dimethyl-uracil > cytosine h.[15]



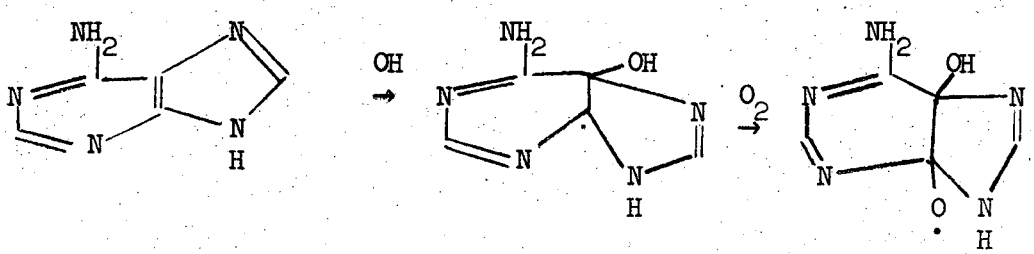
5-hydroxycytosine



uracil glycol

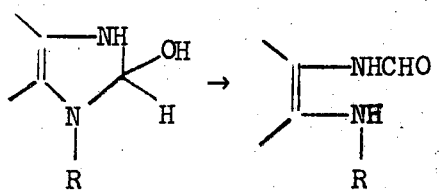
Several authors have shown that purines in aqueous systems are more resistant to radiation-induced degradation than the pyrimidines. This fact is evident in a comparison study of the decomposition of the free bases, as well as in the relative destruction of bases when combined as nucleosides or nucleotides.[16]

The site of radical attack on the purines has been proposed to be the central 4-5 bond of the molecule. This idea has developed from considerations of the known behavior of uric acid and certain other purines to oxidizing agents. Radical attack leads to ring arrangements and ring opening. In the case of oxygenated adenine solutions, this process leads to the production of an organic peroxy radical. Further reactions result in degradation.[17]

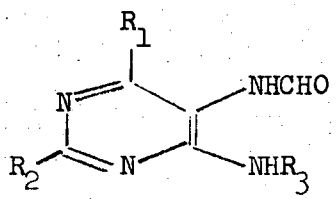


Hydroperoxides, if formed, must be very unstable since such compounds have not been detected. However, the existence of these as unstable intermediates could lead to ring arrangements.[18] Since ring-opening of

the imidazole ring[19] occurs only under oxygen-free conditions, this process most likely results from a reaction of the reducing species, as well as OH radicals with a purine moiety. Production of formamido-pyrimidines requires the net addition of one H and one OH to the imidazole ring. In this way, it is related to the production of dihydro hydroxy compounds from pyrimidines in vacuo. Hence a comparable reaction scheme to that described earlier could be considered. The resulting purine hydro-hydroxy compound then breaks up in the following manner:[20]



Other centers of the molecule have also been found subject to attack. Hems found that irradiation of adenine resulted in the rupture of the 8-9 bond.[21] Irradiation of solutions of guanosine, guanylic acid, xanthosine, inosine, adenosine and adenosine-5'-phosphate leads to the opening of the imidazole ring to form the corresponding 4-amino-5-formadido pyrimidine riboside or ribotide:



The following table indicates the parent nucleoside and nucleotides and the formamido pyrimidine riboside and ribotide formed:

Parent Nucleoside	Formamido pyrimidine riboside (R_3 = ribose)	
Guanosine	R_1 = OH	R_2 = NH_2
Xanthosine	R_1 = OH	R_2 = OH
Adenosine	R_1 = NH_2	R_2 = H
Inosine	R_1 = OH	R_2 = H

Parent Nucleotide[22]	Formamido pyrimidine ribotide	
Guanylic acid	R_1 = OH	R_2 = NH_2
Adenylic acid (5')	R_1 = NH_2	R_2 = H

Similarly, Weiss has reported the formation of 8-oxypurines.[23] Deamination of adenine and the production of ammonia has also been demonstrated.

The above results have dealt with single nucleotide solutions. When the nucleotides are placed together into solution, one finds differences between the purines and the pyrimidines in the extent of their destruction. This is also true for the bases when combined as nucleosides as well as deoxyribonucleotides. According to McCargo, upon irradiation with 200 Kv X-rays of oxygenated solutions (pH 7) containing equimolar quantities (5×10^{-5} M) of the 5'-deoxynucleotides of adenine, guanine, cytosine and thymine, the following molecules/100 ev (the so-called G-values) were obtained: G(adenine) = 0.24; G(guanine) = 0.20: G(cytosine) = 0.34; and G(thymine) = 0.47.[24] These results show that even in

admixture, the extent of destruction of the pyrimidines is nearly twice that of the purines.

The release of free bases, probably as a result of attack on the sugar moiety, occurs at about one-quarter of the frequency at which the bases are destroyed.[25] As a result of the chemical changes in the bases, the glycosidic linkages can undergo hydrolytic fission. However, the actual rates of these hydrolyses are unknown. There is evidence to indicate that the formation of the hydroperoxide on the pyrimidine moiety may not necessarily lead to an immediate break.[26] The free bases arising from the oxidation of the sugar moieties of nucleosides and nucleotides are probably a result of the decomposition of intermediates of the type BS* and BS*P mentioned earlier in this section. However, the stabilities of these types of compounds are unknown. Also unsettled is the site (or sites) of attack by radiation-produced radicals which eventually leads to liberation of free bases. In order to do this, one must consider the reactions of OH radicals which eventually leads to liberation of free bases. In order to do this, one must consider the reactions of OH radicals at various positions in the sugar molecule. In solutions irradiated in the absence of oxygen, one must consider some reaction of the reducing species.[27]

Oxidation of the sugar moiety of the nucleotide may also lead to the labilization of the phosphate groups. In this case, formation of carbonyl groups at carbon atom C(3') (which in the nucleoside-5'-phosphate leads to a slow release of inorganic phosphate) may cause further fragmentation of the polynucleotide chain. This would simply increase the number of

singly-bound phosphate groups. Manifestation of labile phosphate by slow fission to inorganic phosphate can only be expected to occur in the case of attack on a terminal group. This fact has been born out by evidence that very low yields of inorganic phosphate are found. Also, such post-irradiation release of inorganic phosphate has been observed in irradiated solutions of commercial RNA where the number of end groups is large.[28]

It should be noted, however, that the effect of radical action produced by ionizing radiation is lessened due to naturally occurring components of the cell. Components, such as the sulphydryl groups,[29] intermediate by reacting with the radicals.[30] Upon irradiation of synthetic mixtures of DNA and histone, degradation of the DNA component is less than in solutions of pure DNA. Thus, in the synthetic mixtures, the protein acts as a partial protector of the DNA through competition for the available radicals. This realization is important when considering the effects of radiation on nucleoprotein. This particular role of the protein moiety has not been firmly established by both physico-chemical and chemical studies. The protection is not complete however. At doses above 1.3×10^5 rad in 0.02% solutions, base destruction starts to be evident, phosphate can be released on heating, and a visible precipitate is formed.[31]

As the concentration of DNA is increased, the probability of direct radiation effects become important. Electron spin resonance studies have shown that there is in general a high yield of free radicals when DNA or its constituents are irradiated in the dry state. For a given dose, more

radicals are produced in deoxycytosine monophosphate and deoxyguanine monophosphate than in deoxyadenine monophosphate and thymine monophosphate.[32]

The direct effect of ionizing radiation is damage resulting from the passage of ionizing particles through the chromosome. In the 1940's, work by Lea, Catcheside, and associates showed that "breaks" induced by radiation were the result of ionizations occurring in or near the chromosome thread. It was believed that breakage was localized to the near vicinity of the particle tracks.[33] Lea and Catcheside further found that for a given absorbed dose, fast neutrons were more efficient than X-rays. This suggested that single ionizations were insufficient to produce breakage. From a comparison of neutron-X-ray efficiencies, Lea and Catcheside concluded that a minimum of fifteen to twenty ionizations were required for the probability of breaking a chromatid thread 0.1 micron in diameter to approach unity. They found that with the type of hard X-rays that are normally used in chromosome experiments, breakage primarily results from energy dissipated in the densely ionizing "tail" regions of the electron tracks.[34]

More recently, Neary, Savage, and Evens have taken exception to the results and interpretation of Lea and Catcheside. Their experiments indicate an alternate viewpoint is more feasible. Neary proposes that in the cell, just as in simpler chemical and biological units, primary damage to macromolecular targets is produced chiefly by a single energy-loss event. Neary also proposes the following model for the production of chromosome aberrations:

(1) The integrity of any short region of the interphase chromosome (or chromatid) depends on the continuity of a single macromolecule with a diameter of a few millimicrons, presumably a basic double helix of DNA with associated protein.

(2) The primary lesion occurs in such a macromolecule and is normally caused by a single energy-loss event in the macromolecule.

(3) An aberration is formed by the interaction of two chromosome regions each having a primary lesion; interaction is unlikely between lesions caused by energy-loss events more than some critical distance apart, which has been estimated as about 0.2 micron. Here the question of whether the primary lesion is an actual break or not makes little difference to the formal analysis.[35]

It should be noted that Neary and associates accept the Taylor model for the structure of the chromosome. As has been indicated in a previous section, the Taylor model of the chromosome has not be generally accepted as the correct model. Thus this model has yet to be accepted by all investigators. It will be shown in a later section how Neary and his associates have attempted to verify their model. The various models for the formation of aberrations from "breaks" in the chromosome will also be discussed in the later section.

Another effect of radiation impinging upon the chromosome is the breakage of hydrogen bonds. Cox et al. showed by titration studies and also by ultraviolet absorption measurements that hydrogen-bond rupture is an important consequence of the chemical action of ionizing radiations

on DNA in aqueous solutions. It was concluded that from the asymmetrical displacement of the titration curves at 25°C that the hydrogen bonds linking the adenine and thymine bases in the double helix were broken more readily during irradiation than those linking guanine and cytosine.[36]

Hydrogen-bond loss is thought to be the result of breakage of the internucleotide bonds. One explanation of the phenomena is that if, following the chain break, water molecules can somehow "unzip" the double helix of the DNA to some extent, a relatively large number of hydrogen-bond pairs can be broken per single break of the internucleotide bonds. In this way, hydrogen bonding between individual base pairs will be replaced to some extent by interaction between the bases and the surrounding medium (water). This "unzipping" of the polynucleotide strands should be a limited process; Temperely has calculated, from entropy considerations, that in DNA the untwisted length may be of the order of 15 to 20 links.[37]

Before moving on to the topic of the creation of aberrations from "breaks," a more macroscopic observation is in order. According to H. J. Evans, it has long been known that irradiation of cells which are in the early stages of mitosis frequently results in a clumping of the chromosome. The appearance of these clumped chromosomes suggests that the outer surface or matrix of the chromosome has become sticky and, as in mitotically asynchronous cell populations, such stickiness is the first visual manifestation of an irradiation effect upon the chromosomes. This effect has been named the "primary" or "physiological" effect.[38]

This section has dealt primarily with the biochemical details of the

interaction between radiation and the chromosome. It has been pointed out that an indirect effect occurs resulting from the interaction of the radiolysis products of water and the chromosome. It was seen how these products affected the nucleoprotein as a whole as well as the individual bases. It was also shown that a direct effect exists due to ionizations of the radiation particle occurring within or very near to the chromosome. Although early experiments indicated that many ionizations were needed to create a "break" in the chromosome, more recent experiments have shown that only one ionization is needed to create damage in the macromolecule which can then lead to an aberration site. Ionizing radiation was also found to break the hydrogen bonds of the chromosome. One of the visible signs of irradiation has been the clumping of the chromosomes.

This section has dealt with the damage to the chromosome produced by ionizing radiation. The next section will continue the story by describing how the cell can sometimes repair these points of damage in the chromosome.

FOOTNOTES FOR SECTION II

1. K. V. Shooter, "The Effects of Radiations on DNA Biosynthesis and Related Processes," in Progress in Biophysics and Molecular Biology, Vol. 17, Eds. J. A. V. Butler and H. E. Huxley (Pergamon Press, 1967), p. 292.

2. Ibid.

3. P. C. Gooch, M. A. Bender, M. L. Randolph, "Chromosome Abberations Induced in Human Somatic Cells by Neutrons, in Biological Effects of Neutron and Proton Irradiations, Vol. I, Proceedings of the Symposium on Biological Effects of Neutron Irradiation, International AEC (October 7 to 11, 1963), p. 325-342.

4. J. Weiss, Nature, 153, 748 (1948).

5. G. Scholes, "The Radiation Chemistry of Aqueous Solutions of Nucleic Acids and Nucleoproteins," in Progress in Biophysics and Molecular Biology, Vol. 13, Eds. J. A. V. Butler, H. E. Huxley, R. E. Zirkle (Pergamon Press, 1963), p. 61.

6. Ibid.

7. H. J. Evans, "Chromosome Aberrations Induced by Ionizing Radiations," in International Review of Cytology, Vol. 13, Eds. G. H. Bourne and J. F. Danielli (Academic Press, 1962), p. 274.

8. K. V. Shooter, op. cit., p. 291.

9. H. J. Evans, loc. cit.

10. G. Scholes, op. cit., p. 76.

11. The chromosomes were originally found through a staining of the nucleus. It is the ability of the bases to take on the color of the stain,

thus giving the chromosomes a chromophoric character.

12. G. Scholes, op. cit., p. 64.

13. Ibid., p. 70.

14. Ibid., p. 65.

15. Ibid., p. 66.

16. Ibid., p. 73.

17. Ibid., p. 75.

18. K. V. Shooter, op. cit., p. 293.

19. An imidazole is a colorless, crystalline base of the form $C_3H_4N_2$.

20. G. Scholes, op. cit., p. 81.

21. Hems, Nature, 186, 710 (1960).

22. G. Scholes, op. cit., p. 79.

23. J. Weiss, Progress in Nucleic Acid Research and Molecular Biology, 4, 410 (1964).

24. G. Scholes, op. cit., p. 74-78.

25. K. V. Shooter, op. cit., p. 293.

26. G. Scholes, op. cit., p. 79.

27. Ibid., p. 81

28. Ibid., p. 91

29. Some sulphur atoms are contained in the nucleoprotein, sometimes linked to one another in a disulfide link or separated in the sulfhydryl (-SH) form. These groups can sometimes serve to protect the nucleoprotein by being activated by either radiation or by attacking radicals.

30. K. V. Shooter, op. cit., p. 291.

31. G. Scholes, op.cit., p. 294.

32. K. V. Shooter, op. cit., p. 294.

33. H. J. Evans, op. cit., p. 228.

34. Ibid., p. 269.

35. G. J. Neary, "Chromosome Aberrations and the Theory of RBE, Part I," in International Journal of Radiation Biology, Vol. 9, 5 (October 27, 1965), p. 478.

36. G. Scholes, op. cit., p. 94.

37. Ibid., p. 97.

38. H. J. Evans, op. cit., p. 241.

III. CHROMOSOME REPAIR

The classical postulate in the theory of chromosome aberration formation is that radiation induces a large number of breaks or damage points in the chromosomes. The majority of these restitute. Of the remaining breaks, a minority are thought to be involved in exchange and the rest appear as breaks later in the cell cycle at metaphase. With this assumption, chromosome breaks, chromatid breaks, and isochromatid breaks are interpreted as the surviving examples of the primary biological effect which is thought by some to be the breakage (or at least weakening damage) of a continuous interphase chromosome thread.[1] The purpose of this section is to gain an understanding of the various proposed repair mechanisms, how they are thought to affect the number of chromosome aberrations produced, and to compare the repair ability of cells when bombarded with different forms of radiation. The question of how long "breaks" remain open will be discussed, as well as how repair efficiency changes throughout the cell cycle.

An understanding of repair is important as it may bring new light on old calculations such as Lea's in 1955. It is still thought that the majority of primary "breaks" formed after certain forms of irradiation undergo restitution. Many of the older calculations employed in the estimation of the frequency of restitution, however, were found to depend on the observed frequencies of chromosome breaks. There are two things wrong with this type of assumption. First, the true frequency of observed frequencies may well be lower than that found by earlier experiments.[2] Second, it assumes that the chromosomes can repair damage from any

form of radiation, and this, as will be shown in this section, is subject to doubt.

One of the problems with earlier experiments involving observation of the number of chromosome breaks was that it was not known how many breaks were caused by radiation damage. In 1964, for example, Davison, Freifelder, and Holloway reported that their preliminary studies of the size of single DNA chains produced by denaturation of whole bacteriophage molecules showed that few of the DNA molecules existed without breaks in the single chains.[3] The very large single chains are broken extremely easily by shearing forces during processing. This has been evidenced more recently in work by Davison and Freifelder in 1966 which indicated that even if careful techniques are employed, at least 70% of T2 DNA molecules contain no breaks in the single strands.[4]

The sensitivity of bacteria can be altered by changes in incubation conditions during the post-irradiation period. Further studies have shown that variations in sensitivity to cell killing, mutation rate, frequency of chromosome aberrations and effects on metabolic reactions occur within the cell cycle. These observations have led to the hypothesis that cells are able to repair radiation damage.[5] However, as well be discussed later in this section, the degree of radiation repair is found to be very dependent on the type of radiation, the effect observed, and the organism being radiated.

The element of time is an important consideration in the discussion of the relationship between "breakage" and the formation of an aberration. In order to produce an exchange aberration, the coexistence of

two "breaks" in both space and time is thought to be necessary. If this is true, the yield of two-hit aberrations decreases with an increase in exposure time. The rate of decrease is a function of the duration for which "breaks" remain available for rejoining. Also, with a constant dose given at different intensities, the longest radiation time over which no decrease in the two-hit aberration yield occurs is a measure of the time that "breaks" stay open. Similarly, the longest interval between fractionated doses which does not result in a reduction in the aberration yield also gives a measure of the duration for which "breaks" can interact.[6] For example, it was shown in one experiment that the yield of exchanges produced by a given dose of X-rays diminished with increased duration of the exposure. At low intensity, many of the "breaks" restitute before their potential interaction breaks are produced. There is also a limitation on reunion due to the spatial factor. This fact points to the conclusion that many of the induced breaks do not result in the production of aberrations. Earlier calculations developed by Lea and Catcheside and others indicated that the fractions of breaks which restitute, in Tradescantia, for X-rays, fast neutrons, and alpha particles were, respectively, 0.9, 0.9, and 0.5, there being a much higher proportion of incomplete aberrations with alpha-particle radiation.[7] These calculations, however, are no longer used.

There is still some question as to how long breaks remain open. Earlier studies led to a definite disagreement regarding the rapidity of break repair. In one experiment by Sax on Tradescantia, it was estimated that the breaks remain open for 20 to 60 minutes. However,

other experiments by Lea and Catcheside on Tradescantia indicated that the breaks remain open for only 4 minutes. In the later experiments of Catcheside and others with irradiation times of over 30 minutes, it was found that their results did not agree with their theoretical expectations, that is, there were too many interchanges at the low intensities. This discrepancy was not attributed to a high proportion of one-hit interchanges. Instead, it led to the suggestion that two components were involved in chromosome rejoining in Tradescantia: a short-term one measured in minutes and a long-term one measured in hours.[8] Later experiments by Wolff and Luippold in 1956 on Vicia and by N. S. Cohn in 1956 on Allium roots also indicated that there were two sets of breaks. In one group, rejoining took place within about a minute after irradiation. The second group had considerably longer rejoining times. In the Vicia experiments, it was shown that, at a fixed dose, prolonging the radiation exposure from 30 seconds to 1 minute resulted in reducing the exchange aberrations by about 30%, irregardless of oxygen conditions.[9] In 1942, Newcombe suggested that the amount of rejoining decreased with increasing dose and dose rate. The experiments by Wolff and Luippold in 1954 involving fractionation of doses on Vicia roots showed that the time for which breaks remained available for rejoining was dose-dependent and increased with increasing dose. However, the amount or potentiality for rejoining was not dose-dependent.[10]

Before discussing the ability of the cell to repair damage produced by different forms of radiation and the variation of repairability with the stage in the cell cycle, the proposed mechanisms of repair

should be briefly discussed. Bacterial cells were used in many of the early experiments studying repair. For these studies, the formation of thymine dimers[11] in DNA by exposure to ultraviolet irradiation in vivo and in vitro provided the model system for studying in detail the nature and kinetics of the repair reactions. It is now thought that repair or reactivation of ultraviolet-irradiated bacterial cells can be achieved in two ways: by exposure to visible or infrared light (photoreactivation) or by incubation in the dark (dark repair). Both of these processes are thought to be enzymatic in nature and both involve a reduction in the number of thymine dimers present in the DNA. However, the mechanisms involved are thought to be quite different. The term photoreactivation used when referring to cells should not be confused with repair or reactivation of isolated DNA. The latter involves irradiation at a shorter wavelength following the initial exposure. This is thought to be a distinct process which involves photosplitting of the thymine dimer.[12]

Two models are postulated for dark repair of the chromosome: the "cut-and-patch" and the "patch-and-cut" models. The cut-and-patch model suggests that an enzyme excises a short, single strand of the damaged DNA. This gap is enlarged by a nuclease attacking the nucleotides in order. Next the missing bases are replaced by repair replication according to the proper base-pairing scheme of the adjacent strand. The patch-and-cut model, on the other hand, postulates that the proper enzyme cuts the strand of DNA near the defective bases. The repair replication begins at this point and new bases are inserted as the

defective strand is peeled back.[13]

Although the evidence for these two theories is very interesting, the details will not be discussed in this paper. There are three bits of information which should be noted before continuing. First, it was suggested in one of the models that the repair system in the E. Coli bacteria (strain hcr^+) involves at least two enzymes. One enzyme breaks the DNA chain at a thymine dimer or at a damaged base. This forms a starting point from which a second enzyme excises a section of the chain. Ionizing radiation, in contrast to uv irradiation, produces single-strand breaks in the DNA chains. Thus, points would be available for attack by the excising enzyme, and repair would not require the presence of the chain breaking enzyme. To further confirm this hypothesis, Bridges and Munson showed that their hcr^- strain could not repair damage produced by a bifunctional mustard but could repair damage following treatment by methyl methane sulphonate. Although both agents are thought to alkylate bases in DNA, the latter agent, in contrast to the mustard, produces breaks in the single chains.[14] J. H. Taylor claims that the basis for the model hypothesis is somewhat weakened by subsequent studies of reunion of radiation-induced breakage. Chromatid exchanges are thought to occur in the presence of the block by fluorodeoxyuridine. This suggests many of the breaks do not involve DNA and another component, probably protein, is able to bring about a reunion.[15]

Some evidence tends to confirm the idea that the ability to repair damage is dependent upon the dose. It has been found that energy-rich phosphate is probably necessary for rejoining lesions by the growth of

two antiparallel DNA chains. Also, it was shown by Allfrey, Mirsky and Osawa and others that the oxidative generation of energy-rich phosphate occurs within the nucleus. At one time, this process was found only in mitochondria.[16] Moreover, Creasey and Stocken have shown that in certain cells of the mouse, nuclear phosphorylation is very sensitive to radiation. Inhibition of the formation of intranuclear labile phosphates is observed after doses of as low as 25 r. These observations suggest a link with the findings that the chromosome rejoining system is itself radiosensitive and requires energy-rich phosphate for its operation.[17]

The next question is whether these repair mechanisms can be applied to cells other than bacteria. One case has been sited in the previous paragraph in reference to mouse cells. In fact, Shooter claims that there is a considerable body of evidence indicating repair mechanisms are operative in mammalian cells. In most cases, DNA synthesis, cell killing, chromosome damage and mutation are found to be most affected in the S (synthesis) phase of the cell cycle. Irradiation in G1 and G2 is thought to be associated with an increased resistance. Variations in sensitivity within the cell cycle may, in part, be attributable to changes in the extent of damage produced in DNA. This depends upon the degree of condensation of DNA molecules in the chromosomes or to variation of the volume of the nucleus.[18] These possibilities are discussed elsewhere in the paper. The efficiency of repair in mammalian cells is found to vary within the cell cycle. The evidence suggests that repair is more efficient the longer the elapsed time before DNA replication

begins. In HeLa S3 cells, for example, there is a progressive increase in sensitivity through the first part of G2. Also, it has been found that HeLa cells irradiated early in G1 enter the S phase and show no detectable differences in the rate or duration of DNA synthesis. This apparently normal behavior does not mean that all radiation damage has been repaired. During the second cycle of DNA synthesis following division, there is found to be a marked inhibition of the rate of entry of cells into S and of the rate of DNA synthesis when it beings.[19]

The synthesis of the major components of chromosomes is thus restricted to one-half or less of the cell cycle in many cells of higher organisms. Yet, the repair or reunion of broken chromosomes can occur at other stages of the cell cycle. Taylor cites as an example the breakage of chromosomes in the prophase stages in Lilium after a demonstration that DNA replication is no longer possible. These breaks are found to produce chromatid bridges in abundance, which indicates that strands have been rejoined. Other examples are the production of chromatid exchanges in pollen tubes and in the late interphase in in roots. Breaks that are produced before DNA replication in the G1 stage also undergo reunion or exchange before replications.[20]

The next question is whether or not the repair mechanism is applicable to damage produced by ionizing radiation of higher LET, such as neutrons. Here the answer is not so positive. Following a single dose of X-rays, there is found to be a slow return of damaged liver cells of mice toward normal levels. For neutrons, the damage seems to persist almost indefinitely. Cells that have been irradiated by gamma

rays also seem to return to normal given time. It is therefore thought for neutrons there is no chromosomal recovery following any dose of radiation. There is chromosomal recovery after gamma irradiation, the magnitude of which depends on the dose. The reason for this almost certainly lies in the differences in linear energy transfer between the two radiations. As is seen in another section of this paper, gamma rays are found to produce very sparse ionizations. Thus, for gamma rays, ionization events occurring within individual chromosomes will be widely separated in space and time. As mentioned before, it takes two or more such events within a certain region and within a certain time to produce a lasting chromosome aberration. Otherwise the single event can be healed and no permanent damage results. From this it follows that permanent damage resulting from gamma irradiation will follow a multi-hit curve. This is just what is found. On the other hand, the ionization density along a neutron track is so high that if a chromosome is hit at all, two or more closely spaced ionizations will be produced leading to a permanent chromosome aberration. This explains the single-hit curve usually found for neutrons and the lack of chromosome healing.[21]

There are problems in cell division as a result of the lack of repair of the chromosomes. According to Curtis, it would seem entirely reasonable to suppose that a cell containing severe chromosome damage would divide perhaps only once. The daughter cells would be expected to either die or their chromosome damage become invisible. However, in his studies, this appeared to not be the case. One way to explain the slow recovery to normal following gamma irradiation on the basis that a

cell containing severe damage would divide only once and then the daughter cells would either die or their chromosome damage become invisible because there is a low normal cell division in mouse liver. But the neutron-treated mice in Curtis's experiments appeared to show no recovery, which they should if the above explanation is correct. The other explanation for this observation is the possibility that chromosome healing may be continuing in mice even several months after a single dose of gamma rays, whereas there is no healing following neutron irradiation.[22]

One of the problems in comparing the ability of cells to recover from radiation is that the results depend on which type of effect is being observed. For example, cell death or chromosome aberrations may give different results. The ability of yeast to recover is a case in point. In an experiment by J. T. Lyman and R. H. Haynes, it was shown that after heavy-ion irradiation (high LET) of diploid[23] yeast, recovery occurs to the same extent as is observed after X irradiation. On the surface, this fact would seem to contradict the ideas presented earlier claiming that the cell is unable to repair the chromosome after high LET irradiation. The recovery of these cells is thought to occur despite the chromosomal damage, that is, the ability of the diploid yeast to recover suggests that the macromolecular damage is bypassed rather than being directly repaired. Thus the recovery of the yeast is thought to be independent of the chemical nature of the radiation-induced lesions.[24] At present, therefore, evidence indicates that it is most likely that the cell cannot repair chromosome damage produced by highly ionizing radiation such as neutrons. If repair after such extensive damage does

take place, there is very little idea of the mechanism involved.

There is one other interesting point in reference to the effects of post-irradiation handling of the chromosome. Following a single dose of radiation, and especially following neutron irradiation, the percentage of aberrations may increase for some days or weeks. This is a consistent finding; the explanation for it is thought to lie in the fact that cell division is delayed following irradiation. Since the only cells which can be scored are ones which undergo division, a higher percentage of normal cells will undergo division following stimulation by partial hepatectomy[25] and thus the percentage abnormalities will be scored too low. As the damaged cells recover, if they do, they can be forced into division and thus the percentage of abnormalities will rise.[26] However, for a general figure, it was found by A. Marshak that regardless of species or dose, the maximum number of abnormalities detected and scored in the anaphase stage of the mitotic cycle was found at 3 hours after radiation.[27]

The main idea presented in this section is that the cell has been found to be capable of repairing damage to the chromosome following exposure to gamma and X-rays. However, there seems to be a lack of chromosome healing after exposure to neutrons and other densely ionizing particles. Although the mechanism for repair is not known, two models were discussed in this section: the cut-and-patch model and the patch-and-cut model. It is not known how long breaks or damage points remain open. Some investigators have found that the time for which breaks remain open is dose-dependent. In some cells, it was found that repair is more

efficient the longer the elapsed time before DNA replication begins. The next question to be answered is what happens to those chromosomes which experience damage but are not repaired.

FOOTNOTES FOR SECTION III

1. H. J. Evans, "Chromosome Aberrations Induced by Ionizing Radiations," in International Review of Cytology, G. H. Bourne and J. F. Danielli, Eds., Vol. 13 (1962), p. 232.

2. Ibid., p. 229.

3. K. V. Shooter, "The Effects of Radiations on DNA Biosynthesis and Related Processes," in Progress in Biophysics and Molecular Biology, J. A. V. Butler and H. E. Huxley, Eds., (Pergamon Press, 1967), Vol. 17, p. 297.

4. Davison and Freifelder, Journal of Molecular Biology, 16, 490 (1966).

5. K. V. Shooter, op. cit., p. 296.

6. H. J. Evans, op. cit., p. 230.

7. Ibid., p. 229.

8. Ibid., p. 230.

9. Ibid.

10. Ibid., p. 231.

11. A thymine dimer occurs when two thymines in adjacent nucleotides fuse together by the opening of double bonds. This is thought to result in a kink in the DNA thread.

12. K. V. Shooter, op. cit., p. 300.

13. P. C. Hanawalt and R. H. Haynes, "The Repair of DNA," Scientific American, 216, No. 2, 39 (Feb. 1967).

14. K. V. Shooter, op. cit., p. 303.

15. J. H. Taylor, "The Replication and Organization of DNA in Chromosomes," in Molecular Genetics, Part I, J. H. Taylor, Ed., (Academic Press, New York, 1963), p. 96.

16. Mitochondria are stable sausage-shaped structures in the cell which specialize in maneuvering a stepwise series of energy-yielding transactions.

17. H. J. Evans, op. cit., p. 283.

18. K. V. Shooter, op. cit., p. 313.

19. Ibid., p. 314.

20. J. H. Taylor, op. cit., p. 93.

21. H. J. Curtis, J. Tilley, and C. Crowley, "The Cellular Differences Between Acute and Chronic Neutron and Gamma-Ray Irradiation in Mice," in Biological Effects of Neutron and Proton Irradiations, Vol. II, (1964), pp. 149-150.

22. Ibid., p. 151.

23. The diploid state is the chromosome state in which each type of chromosome except for the sex chromosomes is always represented twice.

24. J. T. Lyman and R. H. Haynes, "Recovery of Yeast After Exposure to Densely Ionizing Radiation," in Radiation Research, Supplement 7 (1967), pp. 222-230.

25. Hepatectomy is a method by which cells are pushed into division earlier than normal.

26. Curtis, et al., op. cit., p. 151.

27. A. Marshak, "Species and Tissue Differences Affecting the Relative Efficiency of Neutrons and X-rays in Producing Chromosome

Abnormalities," in Biological Effects of Neutron and Proton Irradiations, pp. 250-251.

IV. FORMATION OF ABERRATIONS

The previous three sections have laid the foundation for a consideration of the formation of aberrations. The first section discussed the prevalent ideas concerning the actual construction of the chromosome and tried to describe the target or particle or molecule being damaged by the radiation. The second section discussed the effects, mostly chemical and physical, that radiation seems to have on the chromosomes. The third section then discussed the possible mechanisms by which some of the chromosome damage can be repaired by the cell and the likelihood of such happening. The next question to be asked is what happens to those parts of the chromosome which are effected by radiation, either indirectly or directly, and are not repaired or recombined with the neighboring section of the chromosome. The purpose of this section is to describe what happens to these weakened or broken areas and the types of aberrations which may result.

Although it is not known exactly what happens to these weak or broken areas of the chromosome, several postulations do exist. This section will first present the classical idea of aberration formation, that of breakage and reunion. It will discuss the reasons for such a hypothesis and will categorize the aberrations types and their modes of production. Several problems with the first hypothesis will be discussed and then an alternate theory of aberration formation will be presented, in this case, the Revell model of exchange. Experimental evidence supporting this alternative hypothesis will be included. Lastly, a discussion of the original area of conflict will take place,

that of the nature of gaps observed in chromosomes which have been irradiated.

Early works in the research of aberration formation seem to have followed one of two models. One of the models postulated that aberrations resulted from single X-ray hits on chromosome parts which were in contact or close association. This was called the "contact-first" hypothesis. The "breakage-first" hypothesis disagreed with this model. It postulated that radiation produces breaks in the chromatin threads which are independent of one another. Some of the breaks were thought to remain unjoined and would appear as simple deletions. The majority of breaks would restitute giving rise to the original configuration or to the formation of a visible aberration, sometimes called illegitimate fusion. The frequency of breaks were thought to be directly proportional to the radiation dose. Aberrations were divided into two categories: (a) those one-hit aberrations which were thought to be unaffected by either altering the dose rate or by splitting the radiation treatment into two doses separated by time intervals; and (b) those two-hit aberrations which were thought to be intensity-dependent. The latter were thought to increase in proportion to the square of the dose.[1] The classical model of breakage and reunion grew out of the second model of aberration formation.

The classical idea became more established as research continued. Many details intrinsic to this idea were investigated. One of the problems considered was an investigation of the three factors that were thought to be involved in producing an observed chromosomal aberration.

These factors were: (a) traversals of a chromosome by an ionizing particles; (b) the probability of causing a break, given a traversal; and (c) given a break, the probability of an observable aberration's being produced rather than restitution of the break.[2] As discussed in an earlier section, a break was originally thought to be a result of several ionizations. Later it was shown that a radiation-induced primary chromosome lesion is normally produced by a single energy-loss event in a structure with a diameter of the order of a millimicron. This lesion can then interact with a similar lesion in the formation of a chromosome aberration.[3] If the break is to participate in an aberration, it must remain open. This idea led researchers into two further areas of inquiry. Firstly, there have been many studies involving the question of how long the break remains open, and, secondly, several quantitative methods have been developed for determining the distance over which breaks can rejoin. Although neither of these parameters have been determined positively, several approximations have been made. These are discussed in both the section on repair and the section on aberration kinetics. The restitution distance was once calculated to range around an average distance of 1 micron in Tradescantia. It is now shown using data on two-break aberrations obtained following fast neutron and X-irradiation of Vicia that this restitution distance is of the order of tenths of a micron.[4] It was also thought that close spatial association is a necessary requirement in order for exchange to take place. This idea led to the postulate that intrachange types of aberrations are favored over interchange types.[5] Theoretical ratios have been developed

concerning these types; however, this subject will not be discussed at this time. Nevertheless, the main idea for the breakage theory remains the same: the coexistence of two breaks in both space and time is necessary for the production of an exchange aberration; breaks that do not combine with other breaks nor restitute to the original form of the chromosome are seen as gaps or deletions.

According to the breakage and exchange theory, the break could occur almost anywhere on the chromosome at any stage of its development. As a result, many different combinations of exchanges are observed at metaphase. The particular type of aberration is postulated in this model to depend both on where the breaks occur and at what stage in the cell cycle the radiation acts on the chromosome or chromatid. In general, however, the types of aberrations induced following irradiation are thought to be of three types. They are classified according to the unit of breakage or exchange which is involved. Chromosome-type aberrations involve both chromatids of a chromosome at identical loci.[6] This is in contrast to those aberrations in which the unit of aberration is the chromatid. The third category of aberrations is called the sub-chromatid aberrations; however, these have not shed much light on the mechanism of aberration production. Two kinds of structural changes are thought to occur within or between chromosomes and chromatids: the exchange, which is thought to be a new rearrangement following the joining of independent break ends; and the simple deletion, which is thought to be the result of a single break within the chromosome or chromatid. The exchange of parts are thought to occur either within the chromosome (intrachange) or

between chromosomes (interchange). There may be a complete exchange in which all of the breakage ends are involved, or there may be an incomplete exchange in which two of the four breakage ends remained unjoined.[7]

In Fig. 1, chromosome-type exchanges occurring within chromosome arms, between chromosome arms, and between chromosomes are shown. A complete exchange within a chromosome arm may result in an aberration that is undetected (an intercalary deficiency). An incomplete intrachange may give rise to what appears to be a simple deletion with or without an accompanying acentric ring.[8] The intercalary deficiencies are sometimes called dot or isodiametric deletions. The interarm intrachange types have been divided into two types according to whether the fusion occurs between proximal-to-proximal or distal-to-distal breakage ends (the U-type) or between proximal and distal ends (the X-type). As can be seen in the figure, the U-type exchanges may result in centric rings and fragments. They are called asymmetrical exchanges. The X-type exchanges may result in either a symmetrical configuration which may not be detected (a complete exchange) or a deletion. In Fig. 1, taken from Evans,[9] C and I refer to complete and incomplete exchange, and Ip and Id to proximal and distal incompleteness.

In Fig. 2, interarm intrachange and simple interchange aberrations are shown. The interarm chromatid exchanges are classified in a similar manner to interarm intrachanges presented in the last figure. Since the structure of the exchange remains until the metaphase stage (due to the pairing of sister chromatids), the chromatid exchanges which are symmetrical are easier to identify. Twelve groups of interchanges are observed

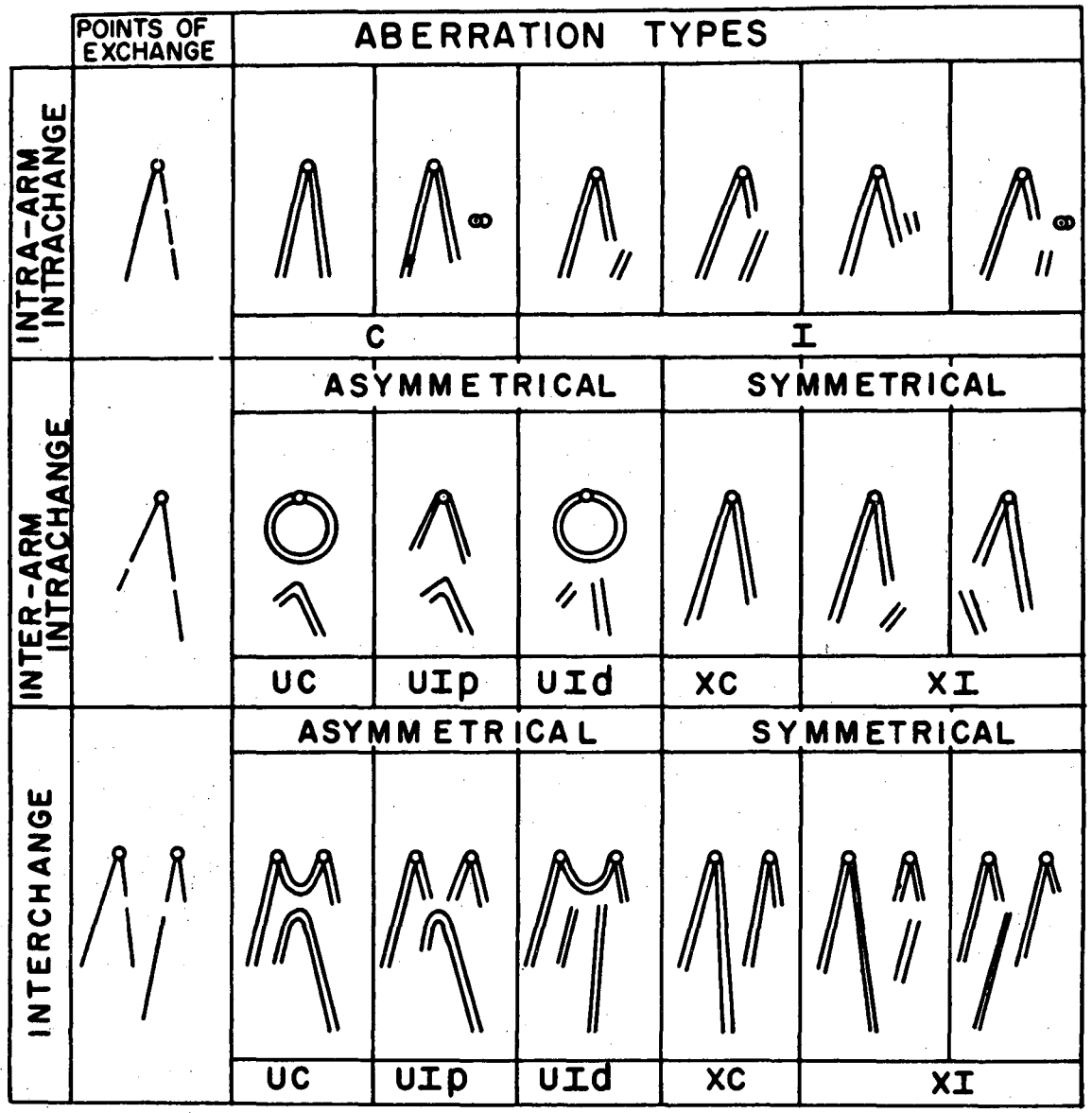


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Fig. 1. Chromosome aberrations resulting from single exchanges. U types refer to cases where fusion occurs between proximal-to-proximal or distal-to-distal breakage ends. X types refer to exchanges between proximal and distal ends. C and I refer to complete and incomplete exchange, and Ip and Id to proximal and distal incompleteness. (From Evans.)

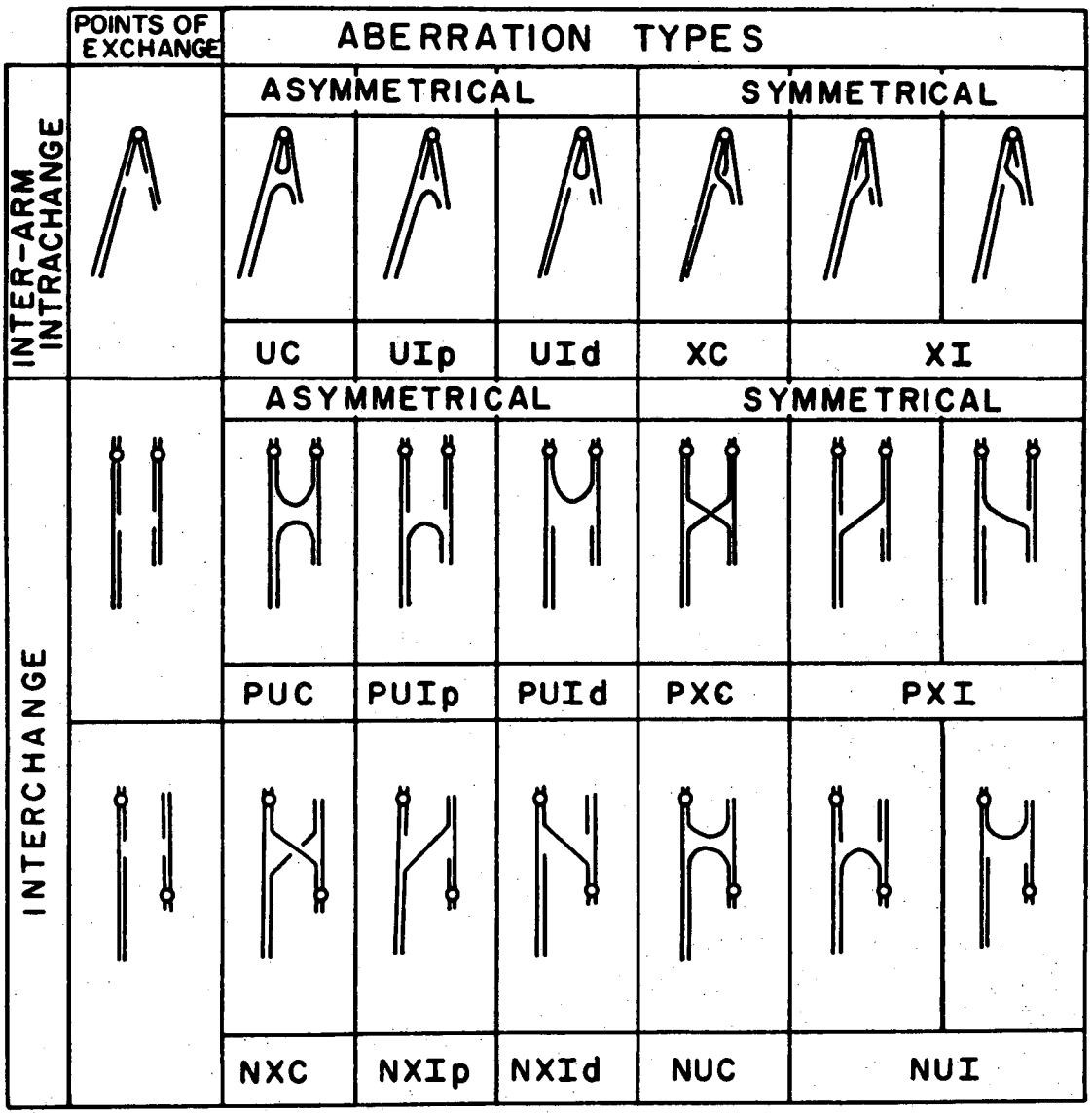

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Fig. 2. Chromatid aberrations resulting from single exchanges between chromosomes and chromosome arms. The symbols P and N represent polarized and nonpolarized chromosomes. U and X are types of exchange which are complete (C) or incomplete proximally (Ip) or distally (Id). (From Evans.)

at metaphase and are classified according to the mode of exchange (U or X), completeness, and the polarity of the chromosomes involved (polarized, P, or nonpolarized, N). The polarity of the chromosomes is thought to be a consequence of the mitotic anaphase movement of the chromosomes to the spindle poles. The rest of the symbols in Fig. 2 are the same as in Fig. 1.

More complex aberrations may occur when more than one exchange takes place at either the chromosome or chromatid level. One of these types is the triradial, which may involve exchange between an isochromatid-type configuration and a simple chromatid break. More complex interchanges may involve the participation of more than two chromosomes.[10]

Some types of aberrations seem to need only one break and others seem to need more than one break. One would thus expect different dose relationships with different types of aberrations. For example, some aberrations--chromatid deletions and chromosome terminal deletions--are thought to be mechanistically the product of single breaks. They demonstrate this by increasing linearly with dose for all types of radiation. The other aberrations--chromosome and chromatid exchanges, chromosome interstitial deletions, and isochromatid deletions--are thought, in this model, to be the result of two breaks combining to give the single aberration concerned.[11] This area of research is discussed further in the section on aberration kinetics. Two predictions, made on the assumption that every break in a chromosome establishes an exchange site, are as follows: firstly, the probability that a chromosome will be involved in a dicentric is directly proportional to its length in the interphase

nucleus; secondly, the number of dicentrics and centric rings per cell will be limited primarily by the number of centromeres. According to Norman and Sasaki, both predictions are confirmed by their data on chromosome-exchange aberrations produced by X-rays in human lymphocytes.[12]

Aberrations are classified in other schemes than that described above. For practical convenience, Neary, Savage, Evans, and Whittle have assigned chromosome aberrations to one of five classes. These are: (1) breaks (terminal deletions); (2) double minutes; (3) single minutes; (4) interchanges ("dicentrics"); (5) intrachanges ("centric rings"). Acentric rings are included in the class of double minutes. They found that a large proportion of these minutes were in fact small acentric rings. The single minutes were not cytologically resolvable as double structures, and double and single minutes comprised all the aberrations sometimes termed "interstitial deletions."[13]

The chromosome is sensitive to aberration production at all stages of the cell cycle. Irradiation of the cells at any stage in the mitotic cycle results in aberrations apparent in the first metaphase stage following irradiation. However, some of the cells do not reach the metaphase stage. For example, X-rays have been found to inhibit the onset of the division states, i.e., the prophase, metaphase and anaphase stages were reduced in frequency. The minimum frequency of anaphases were reached at 3 hours after irradiation. This time coincided with the time found for the minimum frequency of cells remaining normal with respect to chromosome abnormalities. From this coincidence, it was deduced that irradiation blocked the mitotic sequences at the end of the resting stage

and that this was the stage at which the chromosomes were most susceptible to damage by X-rays.[14] One reason that the growth of the cell is stopped is that damaged sites on the single strands of DNA can slow down the process of replication. Numerous studies have been made on the immediate effects of radiation on DNA synthesis. In practically every system studied, it was found that comparatively low doses reduce the uptake of precursor into DNA shortly after irradiation to 40 to 60% of the control level. Further reduction in incorporation requires doses one or two orders of magnitude greater.[15]

Metaphase is not the only stage at which aberrations can be detected. In recent years, a method has been developed for estimating the amount of chromosomal damage present in the somatic cells of mice. It consists of scoring the chromosome aberrations at anaphase in regenerating liver cells. Since liver cells in the normal mouse rarely divide, the method constitutes a way of unmasking the chromosome damage existing in the cell. These aberrations seem to increase steadily with age.[16]

A relationship seems to exist between the aberration structure and the mitotic and meiotic cycles. The breakage mechanism is the same for all aberration types, but the aberration type is differentiated by the unit of breakage or of exchange. The unit of breakage varies as the cell cycle progresses. In one set of studies, uniformity in response was found to be the case at the end of interphase in both mitotic and meiotic chromosomes. In Vicia Faba, the meiotic chromosomes in pachytene gave the same survival curve slopes as those of the mitotic chromosomes at the end of interphase both with X-rays and neutrons. This suggests a

similarity of the chromosome structure at these stages. A common condition to both of these stages which suggested itself was the close proximity of pairs of chromonematic surfaces in the synapsed chromosomes at pachytene, and in the newly synthesized chromosome strands which had just separated at the end of the mitotic interphase. The similarity led the studies into further considerations. If the effective agent producing the chromosome alterations were the electron of the ion pair, closely approximated surfaces if negatively charged would be little affected, while those with a net positive charge would be sensitive. Both chemical considerations and the size of the sensitive volume diameter suggested that the surfaces in question were composed of histones. If this were the case, altering the intranuclear milieu so that the histones would be near their isoelectric point or on the alkaline side, it should lower the sensitivity of the chromosomes to ionizing radiation. Experiments conducted with V. Faba and A Cepa root tips immersed in dilute solutions of ammonia showed that this was indeed the case.[17]

It is not known whether the blockage of mitosis and the production of aberrations are a result of the same phenomenon. For X-rays, the stage of maximum sensitivity is the same both for blocking mitosis and inducing chromosome abnormalities. However, the manner of response to the radiation is different. Inhibition of mitosis is independent of chromatic length and inversely proportional to the number of chromosomes per cell. On the other hand, the frequency of induced chromosome abnormalities is directly proportional to the total chromonematic length of

the chromosome complement.[18]

It is sometimes hard to tell at which stage the cell was irradiated since it has been shown in some species that DNA duplication does not proceed synchronously between all the chromosomes or chromosome regions in a nucleus. Certain of these chromosomes duplicate at much earlier times in the S phase than others. The two diagrams in Fig. 3 (by Evans) indicate the relationship between the type of aberration induced by ionizing radiations as a function of the phase in the mitotic (a) and meiotic (b) cycles.[19]

Early research indicated that treatment at early interphase resulted in only chromosomal-type aberrations, i.e., those produced before the creation of two chromatids. At later stages of interphase, single chromatids of a pair could be broken. It was contended, however, that the degree of subdivision of a chromosome could not be revealed by irradiation because the passage of an ionizing particle could sever several subunits at once. The restitution time is also a factor in this analysis, for another possibility is that a chromosome broken before reproduction may remain open and react with other broken ends after reproduction.[20] Half-chromatid exchanges are induced by radiation only in mitotic prophase and in the stages after pachytene in meiosis. Although the new connections are thought to be strong enough to lead to breakage of chromatids as they stretch in anaphase, the bridges do not persist through the subsequent interphase and usually do not become chromatid aberrations at the next division. After anaphase, the chromatids revert to a state in which they act as if composed of single axial elements in

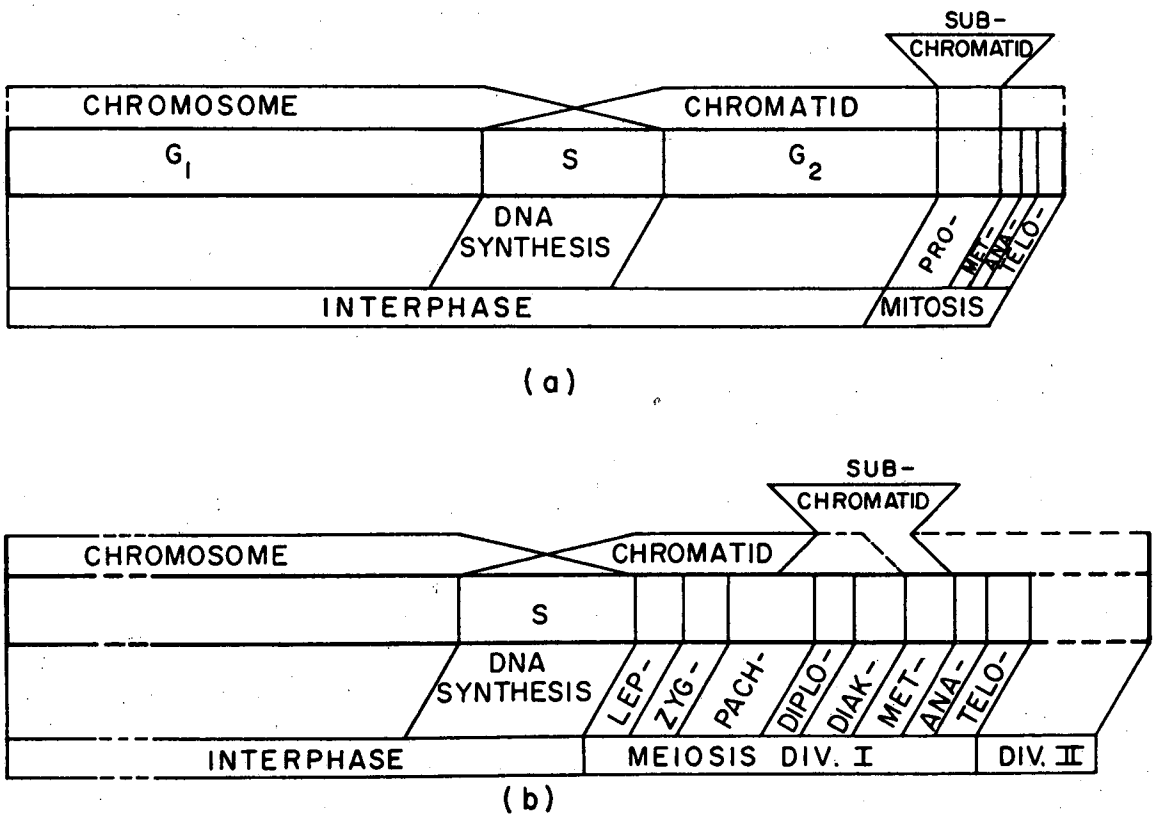

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Fig. 3. Diagrams illustrating the types of aberrations which may occur during the mitotic (a) and meiotic (b) cycles. The type of induced aberration indicates the chromosome structure at each stage in the cell cycle.

breakage and reunion. In the previous prophase, they acted as double axial elements. However, according to Taylor, there is some evidence that they can become double to radiation breakage before DNA synthesis begins.[21]

The breakage-and-reunion postulate flourished until conflicting experimental results began to appear. When the relative frequencies of simple breaks scored by different researchers were compared, considerable differences between scorers were found. This was true even though the same materials and similar radiation conditions were employed. Early work scored an unusually high frequency of breaks. This was due to the inclusion in this class of a group of aberrations called achromatic lesions or gaps. These gaps are thought to be not complete breaks but unstained Fuelgen negative regions in the chromatid. They superficially resemble breaks but are in fact not true discontinuities. This is thought to be true since the continuous nature of a chromatid which contains one or more gaps is evident at anaphase, for gaps do not yield acentric fragments.[22] Gaps will be discussed in more detail at the end of this section.

The classical breakage-and-reunion idea failed to explain another experimental result: The ratio of intrachanges to single breaks was independent of LET. This fact could perhaps be reconciled with the classical hypothesis by an ad hoc increase of the incompleteness factor with an increase of LET. This assumes that the empirical incompleteness factor observed in aberrations is also a measure of the probability of the failure to restitute a primary break (which is postulated to lead to

an observable break). If this were true, however, the incompleteness factor would have to increase continuously with LET; the empirical factor observed did not increase within the range of LET up to 33 kev/micron.[23]

Thus two problems existed with the classical breakage-and-reunion idea. First, the experimental results differed with prediction. Secondly, more complicated aberrations could not be explained adequately. Researchers turned to the idea that the primary event on the chromosome or chromatid may not be a complete break after all, but a weakening of the chromosome thread or a sensitization of the chromosome for aberration. This hypothesis was formulated into an exchange model by Revell and presented in 1959. This alternative hypothesis assumes that a primary event of a temporary nature is instantly produced by the ionizing particle when it crosses the chromatid. Each such event decays unless another is available within a short time and distance to react with it. Such a reaction will, according to this theory, stabilize a pair of primary events in a secondary stage of associated change called exchange initiation. Such secondary sites are predisposed to actual exchange but have not yet reached it. Thus, with this hypothesis, the transient property of each primary point of damage to enter into an exchange initiation does not consist in a break staying open; it is simply a tendency to form some sort of association with another point of damage.[24] It is thought by Revell that all chromatid aberrations are the result of exchange. Thus chromatid breaks and isochromatid breaks are believed to result from interchange. Exchange is thought to occur between chromatid regions which are linearly separated but are brought

into close proximity due to coiling or looping of the chromatids. It is also thought that a large proportion of the intrachanges involve exchange within loops which are small enough to be unraveled during chromosome contraction at mitosis. This looped relation would not be evident at metaphase. In some of the isochromatid aberrations which show sister reunion, the achromatic lesions (indicative of points of exchange) are thought to be sometimes eccentrically placed. This results from an exchange within a loop; the larger the loop, the greater is the relative displacement. In this theory, then, the so-called simple chromatid breaks are really thought to be incomplete intrachanges. In this way, the true chromatid break frequency would be as much as ten to twenty times less than was previously thought.[25] The small intrachanges would unravel and disappear during chromosome contraction to metaphase.

The exchange mechanism is most easily seen in Fig. 4 (by Revell). The aberrations seen at metaphase are associated with a chromatid rearrangement, either within a chromosome or between chromosomes. The complete forms of exchange are shown on the left and the incomplete forms on the right. The four types of intrachange in the lower part are shown in their earlier states (with chromatids completely paired) and as they appear at metaphase (after the chromatids have contracted and so lost their paired relationship within the intrachange).[26]

The support for this hypothesis has come from many corners. Most of the support has resulted from accurate predictions of aberration-type ratios according to the exchange hypothesis. One test of the hypothesis was based on two assumptions: (1) that the four types of intrachange

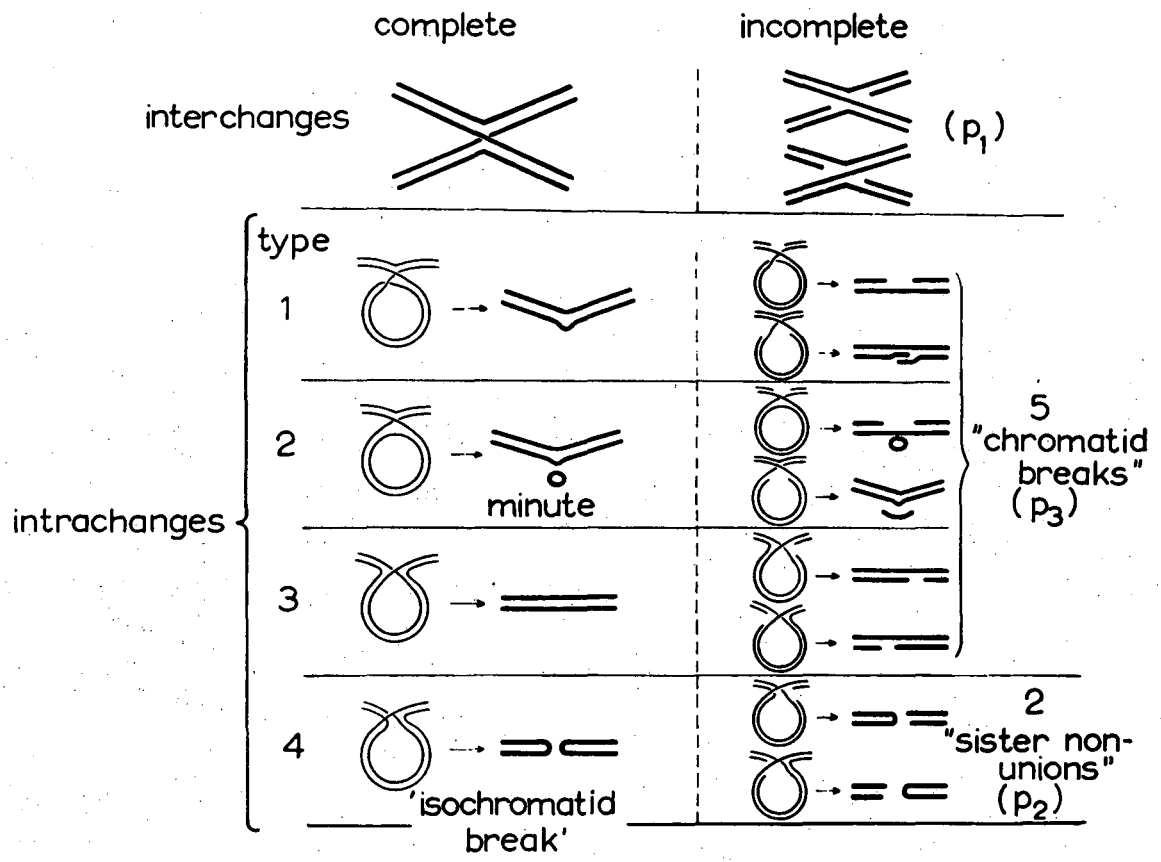

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Fig. 4. Diagram illustrating Revell's exchange hypothesis for chromatid aberrations. The complete forms of exchange are shown on the left and the incomplete forms on the right. The four types of intrachange in the lower part are shown in their earlier states (with chromatids completely paired) and as they appear at metaphase (after the chromatids have contracted and so lost their paired relationship within the intrachange.

are induced with equal frequency; and (2) that the likelihood of incompleteness is the same for all exchanges (both inter- and intra-). Thus the eight incomplete forms of the four intrachanges would be expected to occur with the same frequency. With these assumptions, the proportion of chromatid interchanges which are incomplete should equal the proportion of isochromatid discontinuities which show either proximal or distal sister nonunion. These assumptions also predict that the frequency of single chromatid discontinuities should be only 2.5 times the frequency of sister nonunions of isochromatid discontinuities. This is true since single chromatid discontinuities include all the incomplete intrachanges of types 1 and 3 plus half those of type 2 (the other half being open ring minutes)--thus 5 types--and the sister nonunions are of type 4. Although there is still some doubt as to the precise validity of the two assumptions, these predictions have been pretty much verified by Revell[27,28] and by Savage, Preston, and Neary.[29] It is also predicted using these assumptions that the ratio of the total number of minute rings (2 + 2a + 2b) to the sum of the total number of incomplete (sister nonunion) isochromatid breaks (4a + 4b) and the total number of complete isochromatid breaks (4) should be less than one. This was also found to be true for most cases by Savage, Preston, and Neary.[30]

A significant part of the whole discussion lies in a comparison of the dose administered with the rate of aberration formation. If single ionizations cause chromosome breaks in the classical manner, the original lesions would increase only linearly with dose. Even then, according to the classical breakage theory, many of these breaks would restitute or

form aberrations. However, it is found that the observed points for single breaks fall close to the curve for intrachanges and do not fit the curve expected for primary lesions.[31] In Revell's experiments, it is found that the true chromatid discontinuities increase to the 1.7 power of the dose. It is then hard to see how these chromatid discontinuities are the survivors of a larger number of chromatid breaks increasing only linearly with dose.[32] This LET dependence suggests that breaks are not residual primary lesions but simply a type of intrachange as proposed in the exchange hypothesis.

The verification of the exchange theory is, however, not yet complete. There are some problems with radiations of higher LET. It has been found that the ratio of single breaks plus incomplete intrachanges (excluding incomplete minute rings) to incomplete isochromatid aberrations (expected to equal 2.5 on the exchange hypothesis) is lower for alpha particles than for protons, although it did not vary systematically with dose or gas condition. Similarly, the ratio of intrachanges of type 2 (including isolated minutes) to isochromatid aberrations, expected to be not greater than 1, varied little with any factor other than LET.[33] It is not known yet whether this would mean too few "breaks" or too many incomplete isochromatid aberrations, or too many "minutes" or too few isochromatid aberrations are being scored. It is thought, however, that the displacement of this data for alpha particles is attributable to an excess of incomplete isochromatid aberrations. This leads to a low value for the first ratio. Nevertheless, this data cannot be accounted for by Revell's hypothesis as it now stands with its present

assumptions.[34]

In view of the experiments which have tended to verify the exchange hypothesis, it should be noted that several ideas that are included in the exchange hypothesis that are precluded by the breakage-and-reunion theory. First, there is no theoretical imposition to identify all forms of recovery with chromosome restitution. Instead of the breaks restituting before the end of the recovery time, various forms of recovery might occur at any stage up to that at which the exchange itself becomes structurally established. There is no reason in this theory to assume that exchange initiations must be irreversibly destined for exchange realization. Second, according to the exchange theory, the ionizing particle need not always break the chromatid; the particle need only affect the chromatid in some manner such that the chromosomes in the nuclear environment may perform the later work of exchange. The radiation only minutely predisposed them to do so. Third, the two processes of decay of the primary event and the association of pairs of such events in an exchange initiation may, in this theory, be of different chemical natures. Thus one of these processes may be affected by radiation while the other is not. The breakage-and-reunion idea, however, supposes that the two processes are of the same chemical nature since decay of the primary event is thought to be a rejoining of the chromatid segments, and exchange is thought to be a joining of different chromatid segments. Thus the exchange theory eliminates the notion of legitimate and illegitimate reunion.[35]

Earlier in this section, it was mentioned that one of the

contradictions that led Revell to the exchange hypothesis involved the nature of gaps seen in the chromosome. In earlier studies, gaps were included in the scoring of chromatid breaks. Later, however, it was found that these gaps were not really true breaks. They were, instead, unstained Fuelgen negative regions in the chromatid. However, it has been found that there are several types of gaps that are seen in different ways. Sometimes gaps are found to be thin, nonstaining zones, which stretch across the complete diameter of the chromatid thread, sometimes in association with exchange. They appear to mark the points at which exchange took place. Other gaps are wedged shaped and do not traverse the whole diameter. Sometimes the gaps look like secondary constrictions which are normally associated with the nucleoli. Some of the gaps occur in pairs at identical loci on sister chromatids. Sometimes gaps in nonhomogeneous chromatids may be paired and appear in close association at metaphase. In contrast to true chromatid aberrations, these gaps can be induced in prophase nuclei but are most frequent in those cells which are irradiated at the end of interphase. Although it is not known exactly what produces these gaps, there are several explanations for their occurrence. First, the gaps seem to represent single-hit effects on the chromosomes, as they seem to follow the 1.07 power of the dose.[36] The gaps are also found to be transient. Recovery occurs for few gaps are seen in the second and succeeding mitoses following irradiation. The gaps might be the result of localized despiralization of the chromatids, or they may represent loss or depolymerization of the DNA. It should be noted that the despiralization idea would fit very well with

the repair mechanism proposed by Hanawalt and Haynes (see the section on repair). On the other hand, the depolymerization idea would seem to fit the evidence of Bloom and Leider where they observed DNA-steresis or paling of the irradiated site.[37] The gaps could possibly represent points of damage at which exchange was not realized.[38] This conjecture is supported by the observation that the presence of oxygen during gamma irradiation enhances the frequencies of gaps by about the same amount as for chromatid structural changes.[39]

In summary, two hypotheses for the mechanism of aberration formation have been presented in this section. The breakage-and-reunion model proposes that radiation acts to break the chromosome strands. The loose ends then, if they are not restituted, may link to other loose ends which are created in the same period of time in the same region of space. The exchange model proposes that radiation creates weak points in the chromosome which may interact with other nearby weak points. Although recent evidence tends to support the latter model, neither hypothesis has been verified <u>in totale</u> to date.

The question now arises which mechanism for aberration formation to utilize for the remainder of the paper, the breakage-and-reunion model or the exchange model. However, since this conflict is only apparent when the actual numbers of aberrations of various sorts are listed, the problem is somewhat hidden. Therefore, for the sake of convenience, the term "break" is used throughout the paper, but it should be realized that this could refer instead to a primary event in the chromatid that may not result in an actual break, but in a site for possible exchange. With

this in mind, the paper continues with a consideration of the effect of the linear energy transfer of the radiation particle.

FOOTNOTES FOR SECTION IV

1. H. J. Evans, "Chromosome Aberrations Induced by Ionizing Radiations," in International Review of Cytology, Eds. G. H. Bourne and J. F. Danielli, (Academic Press, 1962) Vol. 13, pp. 229-230.

2. Alan D. Conger, M. L. Randolph, C. W. Sheppard, and Helen J. Luippold, "Quantitative Relation of RBE in Trandescantia and Average LET of Gamma-Rays, X-Rays, and 1.3, 2.5, and 14.1 Mev Fast Neutrons," in Radiation Research, 9, 542 (1958).

3. G. J. Neary and J. R. K. Savage, "Chromosome Aberrations and the Theory of RBE; II. Evidence from Track-Segment Experiments with Protons and Alpha Particles," in International Journal of Radiation Biology, 11, No. 3, 209 (Nov. 24, 1966).

4. H. J. Evans, op. cit., p. 229.

5. Intrachange types include aberrations with exchanges occurring within the chromosome. Interchange includes aberrations with exchanges between chromosomes.

6. A locus is a particular point on the chromosome.

7. H. J. Evans, op. cit., pp. 222-223.

8. An acentric ring is a small closed chromosome segment sometimes found near the aberrated chromosome. It is composed of the part of the chromosome which has been deleted.

9. H. J. Evans, op. cit., p. 224.

10. Ibid., p. 227.

11. A. L. Conger, et al., op. cit., p. 533.

12. Amos Norman and M. S. Sasaki, "Chromosome-Exchange Aberrations in Human Lymphocites," in International Journal of Radiation Biology, 11, No. 4, 321 (Jan. 19, 1967).

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14. A. Marshak, "Species and Tissue Differences Affecting the Relative Efficiency of Neutrons and X-Rays in Producing Chromosome Abnormalities," in Biological Effects of Neutron and Proton Irradiations, p. 251.

15. K. V. Shooter, "The Effects of Radiations on DNA Biosynthesis and Related Processes," in Progress in Biophysics and Molecular Biology, Eds. J. A. V. Butler and H. E. Huxley, (Pergamon Press, 1967) Vol. 13, p. 312.

16. H. J. Curtis, J. Tilley and C. Crowley, "The cellular Differences Between Acute and Chronic Neutron and Gamma-Ray Irradiation in Mice," in Biological Effects of Neutron and Proton Irradiations, Vol. II, p. 147.

17. A. Marshak, op. cit., p. 260.

18. Ibid., p. 251.

19. H. J. Evans, op.cit., p. 247-253.

20. J. H. Taylor, "The Replication and Organization of DNA in Chromosomes," in Molecular Genetics, Part I, Ed. J. H. Taylor (Academic Press, 1963), p. 90.

21. Ibid., p. 92.

22. H. J. Evans, op. cit., pp. 232-233.

23. G. J. Neary and J. R. K. Savage, op. cit., p. 218.

24. S. H. Revell, "Evidence for a Dose-Squared Term in the Dose-Response Curve for Real Chromatid Discontinuities Induced by X-Rays, and Some Theoretical Consequences Thereof," in Mutation Research, 3, 51 (1966).

25. H. J. Evans, op. cit., p. 234.

26. S. H. Revell, op. cit., p. 35.

27. S. H. Revell, Proceedings of the Royal Society of London, Ser. B, 150, 563-589 (1959).

28. S. H. Revell, Mutation Research, 3, 34-35 (1966).

29. J. R. K. Savage, R. J. Preston, and G. J. Neary, "Chromatid Aberrations in Tradescantia Bracteata and a Further Test of Revell's Hypothesis," in Mutation Research, 5, No. 1, 47-56 (1968).

30. Ibid.

31. G. J. Neary and J. R. K. Savage, op.cit., p. 218.

32. S. H. Revell, Mutation Research, 3, 50 (1966).

33. G. J. Neary and J. R. K. Savage, op. cit., p. 214.

34. Savage, Preston, and Neary, op. cit., p. 54.

35. S. H. Revell, Mutation Research, 3, 52 (1966).

36. Ibid., p. 34.

37. William Bloom and Robert J. Leider, "Optical and Electron Microscopic Changes in Ultraviolet-Irradiated Chromosome Segments," in Journal of Cell Biology, 13 No. 2, 214 (1962).

38. H. J. Evans, op. cit., p. 240.

39. S. H. Revell, Mutation Research, 3, 48 (1966).

V. LINEAR ENERGY TRANSFER CONSIDERATIONS

Most of the paper, so far, has concentrated primarily on the biological aspects of the problem. Chromosome structure has been discussed, as well as the breakage, repair, and aberration formation of the chromosomes. The purpose of this section is to introduce a quantity which will relate more to the radiation forms. This quantity, the linear energy transfer of the radiation particle or particles, will allow a comparison to be made between the radiation particle and the damage it creates. This section will first define the linear energy transfer coefficient and then discuss how its magnitude is determined. The implications of a high LET will be discussed as will experimental trends that have been observed.

The linear energy transfer coefficient is useful in the consideration of the effects of radiation because it indicates the quality of the radiation particle. In order to predict the probable damage to the cell as the particle traverses its volume, it is necessary to know how much energy is deposited along the particle track. At the molecular level, the basic index of radiation quality is the number of energy-loss events per unit length of the track of an ionizing particle, usually expressed in units of kev/micron. This is the linear energy transfer.

The average energy expended per primary ionization in a gas is thought to be between 100 and 110 ev. In a condensed medium, where a clear distinction between excitation and ionization processes is not possible, the average energy lost by the primary particle in an inelastic collision is approximately 60 ev. Whatever the precise value may

be in a given case, the average energy lost per collision is large when compared with the energy of chemical bonds. The energy-loss events are believed to occur independently of each other. As a result, the average size of the event is independent of the number per unit length. Therefore the mean rate of energy loss per unit length of a track, the LET, is proportional to the mean number of events per unit length.[1] The LET of a particle is also dependent upon its charge (if any) and its velocity or energy.

There are two ways to determine the average LET of radiation in a medium. In some cases, the two methods give different values. One method is to plot the LET along the particle's total track length, from beginning to end; the average LET for this plot is the track average. The energy average LET is derived from a plot of LET against the energy of the particle, from its initial energy to its final (zero) energy. The averages for the tissue as a whole would then be determined by summing and averaging, according to their contribution to the total track length or to total energy, the track average or energy average LET's from all particles of all energies. One problem with these methods with neutrons is that the track average and the energy average LET's from the D,T neutrons will be quite different. This is due to the large energy but small track length contribution made by the densely ionizing heavy-particle component.[2]

Often the average LET's for the various radiations are employed in a comparison with the relative biological effectiveness (which is dealt with in a later section). The difference in RBE's may be a result of the

is neutrons rather than X- or gamma-rays. Rather, it is the more fundamental physical concept of LET, which is in turn related to neutron energy.[5] The range of average LET's obtained from fast neutron beams employed so far in biological experiments is from 8 kev/micron to 48 kev/micron in tissue, or higher. This difference in average LET within the fast neutron range, and hence the expected difference in sensitivity, is equal to or even greater than the difference between X-rays and energetic fast neutrons.[6]

For an idea of the range of LET's for several radiations, consider Table I.

There have been some attempts to find the distribution of doses due to the actions of neutrons in biological systems. One such case is that of 14 Mev neutrons interacting with Tradescantia tissue. The ionization generated by these D,T neutrons is thought to consist of two different LET components. Of the total dose, 70% is thought to be due to hydrogen recoil protons with low average LET (8.5 kev/micron, track; 16 kev/micron energy). Thirty percent of the total dose is thought to come from elastic and inelastic reactions with carbon, nitrogen, and oxygen yielding heavy particles of high average LET (142 kev/micron, track; 222 kev/micron, energy).[7]

Several trends are noted with increasing or decreasing LET. These trends are often used in testing the various hypotheses for the mode of creation of an aberration. As a result, the information in the following paragraphs should take into consideration the section on the mechanical creation of aberrations.

Table I. Track average and energy average LET's (kev/micron) in Tradescantia wet tissue exposed to gamma-rays, X-rays and neutrons.[7]

	γ-rays	X-rays	Fast neutrons				
			D,T (14.1 Mev monoenergetic)			DD	Cyclotron
Average	(Co^{60} 1.17, 1.33 Mev)	250 Kvp, Hul, 1.45 mm Cu	From p^+ separately	From heavy particles separately	From all particles combined	2.5 Mev	Av ~ 1.3 Mev
Track	0.270	2.6	8.5	142	11.8	31.2	27.5
Energy	0.324	~2.6	16	222	75.1	44.2	51.0

One observed trend with LET is the aberration production per unit dose increases with the track average LET.[9] It should be recalled here that there are two explanations for gaps found in chromosomes: (1) some gaps are due to a lesion in one strand of the chromosome which may lead to an aberration through an exchange process with another lesion; or (2) other gaps result from paired lesions, one in each strand of the DNA molecule. The ratio of aberrations to gaps is found to increase with increasing LET, though incompleteness in aberrations is greater at the top end of the LET range normally examined. These facts indicate that on the classical breakage-reunion hypothesis, gaps are even less likely candidates than the true breaks (terminal deletions) for the role of residual primary breaks.[10] Further results give new insights into the role of gaps.

When the yield of gaps per unit dose in air is plotted against LET on an arithmetic axis, the plot lies on a curve with a finite intercept on the ordinate axis. The curve rises at first in nearly linear proportion to LET. At higher LET, the curve falls progressively below the linear relation. Thus the yield of gaps may be thought to consist of two components. The first is evidenced by the finite value of total yield per unit dose at zero LET. If it is present as a nearly constant background at all values of LET, the remaining component would then have a similar LET dependence to that of the true aberrations themselves. This leads to a possible interpretation of the second component: This type of gap might be a by-product of aberrations that have failed. Here the gap would be the residual expression in a chromosome of an unsuccessful attempt at exchange with another region of damaged chromosome. A few gaps were also found to

be observably associated with aberrations. Although not enough were found to be statistically viable, the yield per unit dose increased approximately in proportion to LET. However, the LET dependence of the second component could also be explained in another manner if this type of gap were the result of two primary lesions close together in the same chromatid, each lesion being produced by a single energy-loss event along the same particle track. In either case, in target theory, the shape of the LET dependence of the second component of gaps would be determined by the size of the formal targets for primary lesions. This would be irrespective of whether the two targets were in two different chromosomes or closely adjacent in the same chromosome.[12] Further studies into the creation of gaps could also shed light on the argument on chromosome structure, single-stranded or multiple-stranded, since the mode of formation of a gap would largely depend on the orientation of DNA fibers along the chromosome strand.

The principle cause of the pronounced LET dependence with chromosome aberrations and possibly also cell killing is the fact that pairs of macromolecular targets must be damaged.[13] Several studies with fast neutrons and with alpha particles have tended to show that all aberrations, including those which were two-hit with X-rays, increased linearly with dose and were independent of intensity. These results indicate that with these radiations the two breaks involved in the exchanges are not independently produced. They result from the passage of a single ionizing particle. The absence of any two-hit effects with the densely ionizing particles implies there is no free exchange between breaks. Only those breaks which are

close together are able to participate in exchange.[14] Stated in another way, of the particles that traverse one chromatid or one strand of the chromatid, a certain proportion, independent of LET but dependent on the distance chromatids are apart, will traverse the other also; apparently the proportion of these that break both, rather than only one chromatid or one strand of the chromatid, increases with LET. This increases isochromatid production at the expense of chromatid-deletion production.[15] Thus, among those aberrations that are the result of two breaks--chromosome and chromatid exchanges, chromosome interstitial deletions, and isochromatid deletions--the proportion made up by the dose-squared term, the so-called "two-hit" aberrations, diminishes with an increase in LET. Also, the ratio of isochromatid to chromatid deletions is found to increase with increasing LET.

This line of thinking is borne out by experiment. Conger et al. in 1958 found that chromatid deletions, although present in large numbers, linear with dose, and accurately measurable, showed an interesting compensatory relation with isochromatid deletions as LET increased. It was observed, as postulated above, that the ratio of isochromatid to chromatid deletions increased with increasing LET. For their first experiment, the isochromatid/chromatid ratio increased from 0.36/1 for X-rays (at 56 r, the dose that resulted in 50% normal cells) to 0.78/1 for D,T neutrons, to 1.02/1 for D,D neutrons. The second experiment showed that it increased from 0.43/1 for gamma-rays to 0.55/1 for X-rays.[16]

As has been discussed in the section on the formation of aberrations, exchange between chromatids are also possible. In studies with

pollen tube mitosis, interchanges are found to be much less frequent than isochromatid aberrations. The scoring of incompleteness in these interchanges is found to be particularly difficult. It is considered, therefore, that these results agree with the generalization that incompleteness is greater at the high LET values of natural alpha particles than for the medium LET range of protons (or fast neutrons) and low LET range of X-rays and gamma-rays.[17]

It can be seen, therefore, that LET studies have the possibility of shedding much light on chromosome structure (distance between strands, single-stranded versus multi-stranded structures, etc.) as well as on the relative biological effect of neutrons compared to other forms of radiation (this is dealt with in a later section). Part of the problem with previous studies of the effects of radiation on the cell is that only part of the aberration types were scored in the determination of the damage produced by radiation. The practical result of LET studies in this case then is that it shows that the sum of chromatid plus isochromatid deletions, rather than either alone, is the useful measurement for the LET-RBE comparisons.[18]

FOOTNOTES FOR SECTION V

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2. Alan D. Conger, M. L. Randolph, C. W. Sheppard, and Helen J. Luippold, "Quantitative Relation of RBE in Tradescantia and Average LET of Gamma-Rays, X-rays, and 1.3, 2.5, and 14.1 Mev Fast Neutrons," in Radiation Research, 9, 531 (1958).

3. Ibid., p. 541.

4. Ibid., p. 530.

5. Ibid., p. 535.

6. Ibid.

7. Ibid., p. 532.

8. Ibid., p. 540.

9. Ibid., p. 533.

10. G. J. Neary and J. R. K. Savage, "Chromosome Aberrations and the Theory of RBE. II. Evidence from Track-Segment Experiments with Protons and Alpha Particles," in International Journal of Radiation Biology, 11, No. 3, 218 (1966).

11. Ibid., p. 219.

12. Ibid.

13. G. J. Neary, op. cit., p. 478.

14. H. J. Evans, "Chromosome Aberrations Induced by Ionizing Radiations," in International Review of Cytology, Eds. G. H. Bourne and J. F. Danielli, (Academic Press, 1962), p. 228.

15. Conger, et al., op. cit., p. 536.

16. Ibid.

17. G. J. Neary and J. R. K. Savage, op. cit., p. 214.

18. Conger, et al., op. cit., p. 536.

VI. RBE CONSIDERATIONS

The previous sections have reviewed the theoretical considerations of radiations in general, and neutrons in particular. The effects of the radiation as it passes through the biological material has so far been discussed in terms of the types of chemical breaks it induces, the types of aberrations it creates and how the type of radiation determines what kinds of effects will result. This section is added to concretize the effects by quantitatively comparing the biological effects of neutrons as opposed to other types of radiations. It also discusses how the energy of the neutrons affects the biological damage. The normally used parameter for comparing the biological effects of different radiations is, as would be expected, denoted the relative biological effectiveness.

The definition of relative biological effectiveness parameter attempts to create a fine structure quantity which will correlate the effects of different forms of radiation. The relative biological effectiveness, hereafter called the RBE, is defined normally as the ratio of the energy imparted to a unit mass by therapy X-rays to the energy imparted to a unit mass by the given type of radiation in order to produce the same effect. For example, suppose for a particular effect the RBE of neutrons to therapy X-rays is 4:1. This means that the dose of neutrons is one-quarter of that needed to produce the same effects with X-rays. The dose of radiation is in turn defined and measured in several ways. According to Alexander, the first well-defined physical unit for measuring X-ray and gamma-ray dosage was the roentgen--named after the discoverer of X-rays. It is defined as the dose of radiation which produces 2.1×10^9

ion pairs in a volume of 1 cubic centimeter of air. This quantity can be converted into an energy unit by knowing that 34 ev of energy are transferred to the gas every time an ion pair is formed. Part of this energy is used to form the ion pair and part to excite other molecules. Thus, exposure of water or tissue to 1 roentgen results in an uptake of almost 100 ergs per gram of water or tissue irradiated.

The number of roentgens per minute given off by an X- or a gamma-ray source is usually measured in an ionization chamber. Here ions of opposite sign are attracted towards two plates charged respectively positive and negative. The electric current in this chamber is then a direct measure of the number of ions produced. The roentgen, because it is defined in terms of an ionization current, cannot, however, be used to describe the dose received by exposure to particulate radiation such as alpha or beta rays. Nor are methods for measuring dose in roentgens (i.e., number of ionizations in a fixed volume of gas) applicable to the very high voltage X-ray machines now used in modern radiotherapy. As a result, a new unit has been introduced. It is called the rad and is defined directly in terms of energy and absorption. One rad is defined as the quantity of radiation which will result in the absorption of 100 ergs of energy per gram of the irradiated material. It is applicable to all types of ionizing radiations. For comparison purposes, the rad is very similar to the roentgen for the exposure of tissues to X-rays. In the latter case, irradiation of one roentgen leads to energy uptake of 97 ergs per gram.[1] With these units in mind, the RBE will be the ratio of doses required to produce the same amount of damage.

One of the problems in using the RBE parameter is that there is no single value for a particular type of radiation. The purpose of this section is to discuss what factors determine the value of the RBE in any particular experiment on any particular organism. It will be seen that the RBE of a particular radiation varies from one type of biological damage to another. The RBE is also influenced by the condition of exposure and by the cell characteristics of the organism involved. For example, the RBE determined by lethal experiments on cells will be very much different from the RBE determined by radiation sickness experiments. In fact, the RBE is even found to be dependent upon the particular type of chromosomal aberration created. Nevertheless, since the RBE for acute effects and chronic effects are around the same level as the RBE for chromosomal aberrations, it is thought that these effects can be explained on the basis of chromosome damage.

Many early attempts to determine the RBE of various radiations led to the search for a simple relationship between the dose of the radiation used and the resulting RBE. Part of the problem with these types of studies is that it appears that there can be no single value for the relative sensitivity to different radiations for the two-break aberrations, at least between X-rays and neutrons. Two break aberrations increase quadratically with X-ray dose and linearly with neutron dose.[2] Although the aberrations seen for 14.1-Mev neutrons are usually qualitatively the same as those induced for X-rays, there is evidence that the chromosome damage due to a single dose of neutrons is about twice as great as the same rad dose of gamma-rays and maybe more for the same rad dose of X-rays. One

reason for this difference in effect may be the ability of the chromosomes to heal partially following even large doses of X-rays. There is no known healing following neutron irradiation. This offers a general explanation for the differences in RBE between different radiations and dosage regimens based entirely on the ability of the chromosomes to heal following X- or gamma-ray irradiation.[3] For example, following a single dose of X-rays, there is a very rapid rise in aberrations followed by a very slow return toward normal. In this case, the increase in aberrations is roughly proportional to the X-ray dose. When mice are given a single dose of neutrons, there is likewise a spectacular sudden increase in aberrations; however, the values stay high for more than a year. This is in marked contrast to the situation with X-rays.[4]

The fact that repair is taking place after radiation doses must be taken into consideration during experiments. Several experiments have been performed comparing the RBE of neutrons as compared to other radiations as a function of the dose rate. Neary and Evans utilized low doses or dose rates in their laboratory. They used as their indicator chromosome aberrations in Tradescantia microspores induced by Co^{60} gamma and fast neutron radiation. In these experiments, they found RBE values for fast neutrons to gamma rays of around 80:1 when the gamma radiation was given over a period of 48 hours. In contrast to this, the expected relative efficiency using exposures of a few minutes to gamma rays would be about 10:1.[5] Similarly, mice, when exposed to chronic gamma-radiation, develop chromosome aberrations faster than the controls. However, these are 25% as effective in shortening the life span as is the same dose applied acutely.

This indicates that partial healing of chromosomes takes place following a very small dose of radiation. Little, if any, healing takes place after large doses.[6] The exposure time and dose-rate considerations are especially important when comparisons are attempted between sparsely and densely ionizing radiations. The majority of the aberrations following sparse ionizing radiations are dose-rate dependent, whereas with the densely ionizing radiations, they are dose-rate independent. Thus such RBE comparisons are difficult since there is no unique relative efficiency value when the aberration yield curves are of different shapes.[7]

As mentioned earlier in this section, several attempts have been made to correlate the level of chromosome aberrations and the dose given to the respective tissue. Similarly, attempts have been made to fit this into a simple mathematical representation. One such study was made by P. C. Gooch, M. A. Bender, and M. L. Randolph at Oak Ridge in 1963. They found that the aberrations produced by neutrons are qualitatively the same as those induced by X-rays. Nevertheless, they found that the type of aberration was a function of dose and radiation type. For example, their curves for deletions corresponded closely with a least-square fit of the data to the model $Y = a + bD$, where Y is the yield of deletions, D is the dose, and a and b are curve constants. Although data for ring and dicentric chromosome production were found not to differ significantly from the linear model, the data fit most closely to the dose-square model $Y = a + cD^2$. Both X-rays and neutrons fitted this type of data. However, the coefficients for the 14-Mev neutrons were significantly different from the X-ray coefficients. The RBE for deletion production for 14-Mev

neutrons was found to be 2.6 (with a coefficient ratio of 0.23/0.09). The RBE for ring and dicentric production was found to be only 1.4 (0.81/0.60).[8] At the time of their article, they only had preliminary results for 2.5-Mev neutrons. Their results were unpredictable as they found a nonuniform response. They felt that the source of the variation was biological and not in the neutron source or the physical dosimetry. In spite of the incomplete nature of the evidence, several tentative conclusions were drawn. The kinetics of the dose response for two-hit aberrations induced by 2.5-Mev neutrons were, superficially at least, linear in the leukocyte system. The RBE for chromosome deletions was found to lie somewhere between 4 and 5. Since the kinetics for rings and dicentrics were found to be different for X-rays and for 2.5-Mev neutrons, they felt that no RBE could be calculated for these types of aberrations.[9]

Neary and Savage also found their results were a function of the types of chromosome aberrations observed. When incompleteness ratios were calculated for isochromatid aberrations at different dose-gas conditions for each radiation quality, no indication of a systematic variation with dose was observed within the dose-range covered. Incompleteness was usually slightly higher in air than in nitrogen for the proton irradiations. The reverse was true for the alpha particles. Incompleteness in isochromatid aberrations was found to be about twice as high for alpha particles as for protons, but there was little difference in interchanges.[10]

Part of the problem with these types of study is that the usual

method of assessing RBE is based on comparing the doses producing matching responses, i.e., equal amounts of effect. At every response level, both single-action and interaction mechanisms contribute to the total response. However, the contribution of interaction mechanisms increases with dose and dose rate, the more rapidly, the less the LET of radiation. Therefore, the RBE based on the total response gives values reflecting the various contributions from the two types of mechanisms, and thus changes with response level.[11]

Investigators are now beginning to compare the efficiencies of the various radiations through the use of LET. It is implicit in the target theory that the different efficiencies of various radiations are not due to qualitative differences between the radiations. Rather, they are due to differences in the rates of energy dissipation along the tracks of the ionizing particles in the tissues (the LET). A striking demonstration that the specific ionization density or the rate of energy dissipation along a particle track (and not the type of particle) is the important factor in determining the RBE for chromosomal breakage was provided by the work of Giles and Tobias in Science in 1954. In these experiments, as interpreted by H. J. Evans, the chromatid aberration frequencies induced in Tradescantia by equal doses of three types of radiation having similar LET values, 30-Mev alpha particles, 190-Mev deuterons, and 100-Kv X-rays, were compared with the aberration yield induced by deuterons having quite different average LET values. The results showed that radiations having similar LET gave similar aberration yields; radiations having different LET gave different aberration frequencies.[12]

According to Conger, Randolph, Sheppard, and Luippold, a turnover of RBE with LET is to be expected on the basis of target theory, but hardly as precipitous as is found with the energy average LET. Breakage of a chromosome is thought to depend on two things: (a) a particle's passing through or at least very close to it (as was discussed in the section on chromosome breakage), and (b) the probability of a break's being produced, given the traversal. The number of chromosome traversals per unit dose, which is directly related to total track length, varies inversely and smoothly with track average LET. The probability of primary breakage is thought to be expected to increase with LET. By an argument involving (1) estimates of traversals, (2) the proportion of primary breaks that fail to restitute and so become observable aberrations, and (3) aberration yield per unit dose, it can be shown that the probability of primary breakage actually increases. Some estimates of restitution frequency, as is discussed in the repair section, show it remains the same for gamma-rays, X-rays and neutrons; for alpha particles restitution frequency actually decreases.[13] In support of this, data obtained from experiments with alpha rays, which have an ion density greater than that of the protons produced by fast neutrons, indicated that alpha particles were more and not less efficient than neutrons. These results were interpreted to show that the probability of breakage following the traversal of a chromatid by an alpha particle was approximately unity. It was then concluded that alpha particles need not always traverse a chromatid thread in order to produce a break, but that breaks may be induced when a particle passes in the immediate vicinity of a

chromosome.[14]

As predicted, chromosomal RBE has been found to increase smoothly with track average LET from gamma-rays to the highest-LET neutrons (1 Mev average energy from the cyclotron). The smooth and considerable increase in RBE in the fast-neutron or gamma-ray comparisons is meaningless unless neutron average LET is considered, at least for chromosomal aberrations. Chromosomal RBE apparently peaks at an average LET somewhat above 50 kev/micron, roughly between 50 to 70 kev/micron. This LET can be obtained with fast neutrons of average energy somewhat less than 1 Mev. At higher LET's, RBE falls and appears to change slowly if at all in the LET range between that of alpha particles and heavy particles from D,T neutron irradiation.[15]

Attempts have been made to find the optimal LET for maximum RBE. At this optimum energy, two of the factors involved in producing a chromosome loss, namely the number of traversals of the arm of the chromosome by a particle track and the probability of a traversal causing a break, would be maximized. One experiment with maize showed that the RBE was highest with the highest energy average LET that they could obtain. This was 72 kev/micron, and the neutron had an energy of 0.43 Mev. It is probable that still higher RBE's might be obtained at average energy LET's above 72, but ultimately the RBE would be expected to decline. This would occur when an ion density is reached at which the decrease in number of traversals of the chromosome becomes more than is compensated for by the increased probability of a break, given a traversal.[16] The relationship observed between the RBE and energy of fast neutrons is similar to the results of

Bateman, et al. on mice. They found a minimum RBE at dose average LET of 85. Conger, et al., on chromosomes of the plant Tradescantia, found a maximum RBE at dose average LET of 50 to 70. The highest RBE values reported for these animal and plant materials were of the order of 10. The low LET responses were found to be curvilinear.[17] Similarly, Neary, et al., were led to the conclusion that neutrons in the energy range of about 0.5 to 2 Mev are more highly efficient in breaking plant chromosomes, relative to X-rays, than had been indicated previously in experiments where the influence of the dose-squared term was minimized. In another experiment, Davies and Bateman found a maximum RBE value of about 40 for 0.65-Mev neutrons compared to 250-kvp X-rays in causing somatic mutations in stamen hairs of Tradescantia.[18] So, in general, it has been found that at low yields with small enough doses, the linear term for the low-LET radiation would predominate over the square term. The RBE of a high-LET radiation relative to a low-LET radiation, instead of increasing indefinitely as the dose was reduced, would tend to a maximum limiting value.[19]

Much to the dismay of those who would like a simple mathematical relationship between the LET and the RBE, other problems arise. It has been found that when an allowance is made for delta-rays, which emanate from the main particle path, the effectiveness per rad is not a simple analytic function of average LET; there is not even a unique relation between effectiveness and LET. For a given LET, the nature of the primary particle has been found to have some influence. Thus the relation between the LET for maximum effectiveness and the thickness of the

formal target, in the target theory, is not a simple one.[20] In their calculations, part of which are considered in another section, Neary and Savage found that the calculated effectiveness per rad continues to increase for LET beyond 165 kev/micron. This was the extrapolated estimate of LET for maximum effectiveness when delta-rays were not considered separately. The physical reason for this continued increase in calculated effectiveness is that the delta tracks are not randomly distributed in space. Instead, they are concentrated around the primary track so that the large local fluences lead to two-track processes between a primary track and one of its delta-rays, or between two delta-rays from the same primary track.[21]

Another problem in the search for a universal RBE, given the particle, its LET, and the dose rate, is that the effect of the radiation is found to change with the tissue. Each tissue is found to display a different pattern of variation. Comparisons of the patterns of variation have revealed that they are not characteristic of the species. This might have been expected from genetic and morphological considerations, i.e., from the identity of the gene complements and similarity of the chromosome morphology in various tissue cells of the same organism. Instead, there has appeared to be a greater similarity between the patterns obtained for chromosomes of similar cell types in different species. Thus, for example, the pattern for the rat lymphoma resembles the mouse lymphoma pattern more than that of the rat carcinoma. One reason for these differences in the relative response of chromosomes is thought to be determined by differences in the functional states of the chromosome during the interphase

period.[22]

One way chromosomes can differ in the various functional states is in the volume they occupy in the nucleus. This was first indicated by the work of A. Marshak in 1937 when he found that the aberration frequency (abnormal anaphases) following X-ray or fast-neutron treatment of cells of mouse sarcoma, Walker carcinoma of the rat, and of root tips of seedlings of tomatoes, Vicia, Pisum, and Allium, "varied directly as the total length of the chromonemata of the somatic chromosome complement" of these tissues. The increased effect which accompanied the increase in chromosome volume was interpreted as being due to an increased target size.[23]

The volume the chromosomes occupy in the cell is thought to be related to the stage of development of the cell. It can thus be expected that the RBE should change with the different stages of development. In one study, for example, H. H. Smith found that the differences in RBE values among leaves were conspicuous and consistent. These leaves were in different stages of development at the time of irradiation. They were found to undergo different numbers of mitoses after irradiation to reach maturity and to produce chromosome sectors of markedly different sizes. His studies have now led him to place the mutation frequency on a per-krad-per-cell basis and to assess the factors that contribute to the quantitative difference in irradiation response in the different leaves.[24] In another study, Deschner and Sparrow irradiated Trillium anthers with both X-rays and thermal neutrons. Although a similar rise and fall in sensitivity due to stage in development was found with both types of radiation, an important observation was made: For X-rays, a 20-fold variation was found between the most sensitive and least sensitive stages; for neutrons,

only a 4-fold range was observed. Although the amount of detectable rejoining with thermal neutrons was less than with X-rays, this difference in rejoining is not enough to account for the differences observed between the two types of radiation.[25]

Another reason for the differences observed in RBE's of various types of cells is thought to be the volume of the cell chromosomes. As discussed in the section on LET, "knock-on" protons produced by fast-neutron irradiation give linear, or near linear, dose-response kinetics. They produce dense enough ionizations to have a high probability of inducing more than one break within a volume where two breaks can interact. One explanation for the difference between Tradescantia and Vicia on the one hand, and the human cells on the other, might be that the volume within which breaks can interact is larger in the human leukocytes than in the plant cells. If this volume were large enough to make it unlikely that one proton track could produce more than one break within it, the resulting dose-effect kinetics for two-hit aberrations would follow the dose-square law. It would seem possible then that such a difference in the volume within which two breaks can interact might be a consequence of the larger number and smaller size of human chromosomes when compared with Tradescantia and Vicia chromosomes.[26]

So, in summary, this section has attempted to show that the relative biological effectiveness of a particular radiation is a rather relative parameter. The RBE has been found to depend on total dosage, dose rate, time until inspection, LET, particle type, types of biological damage observed, stage of cell development, volume of the chromosome,

and type of tissue inspected, just to name a few factors. Estimates for the RBE of neutrons as compared to X-rays have varied from a value around 2 for 14.1-Mev neutrons, to a value of 5 for fission-spectrum neutrons,[27] to a value approaching 100.[28] As one can see, there is no single RBE value for neutrons. It was seen that the difficulty in determining RBE on the basis of chromosomal exchanges or two-break aberrations was that the dose-response curves differed for radiations of different LET and dose rate. Yet, in general, it was seen that the dose-squared term tends to predominate with radiations of low LET (such as gamma rays and most X-rays) and high doses or dose rates; the linear term dominates with high LET tracks in general and at low doses or dose rates.[29]

FOOTNOTES FOR SECTION VI

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3. H. J. Curtis, J. Tilley, and C. Crowley, "The Cellular Differences between Acute and Chronic Neutron and Gamma-Ray Irradiation in Mice," in Biological Effects of Neutron and Proton Irradiations, Vol. II, IAEC (Vienna, 1964), p. 151.

4. Ibid., p. 147.

5. H. J. Evans, "Chromosome Aberrations Induced by Ionizing Radiations," in International Review of Cytology, Eds. G. H. Bourne and J. F. Danielli, 13, 273 (1962).

6. Curtis, et al., op. cit., p. 147.

7. H. J. Evans, op. cit., p. 272.

8. P. C. Gooch, M. A. Bender, M. L. Randolph, "Chromosome Aberrations Induced in Human Somatic Cells by Neutrons, in Biological Effects of Neutron and Proton Irradiations, Vol. I (1963), IAEC (Vienna), p. 336.

9. Ibid.

10. G. J. Neary and J. R. K. Savage, "Chromosome Aberrations and the Theory of RBE. II. Evidence from Track-Segment Experiments with Protons and Alpha Particles," in International Journal of Radiation Biology, 11, No. 3, 214 (1966).

11. H. H. Smith, J. L. Bateman, H. Quastler, and H. H. Rossi, "RBE of Monoenergetic Fast Neutrons: Cytogenetic Effects in Maize," in Biological Effects of Neutron and Proton Irradiations Vol. II, IAEC (Vienna, 1964), p. 234.

12. H. J. Evans, op. cit., p. 270.

13. Conger, et al., op. cit., p. 540.

14. H. J. Evans, op. cit., p. 269.

15. Conger, et al., op. cit., p. 541.

16. H. H. Smith, op. cit., p. 245.

17. Ibid.

18. Ibid.

19. G. J. Neary, J. R. K. Savage, H. J. Evans, and J. Whittle, "Ultimate Maximum Values of the RBE of Fast Neutrons and Gamma Rays for Chromosome Aberrations," in International Journal of Radiation Biology, 6, No. 2, 127 (1963).

20. Neary and Savage, op. cit., p. 217.

21. Ibid.

22. A. Marshak, "Species and Tissue Differences Affecting the Relative Efficiency of Neutrons and X-Rays in Producing Chromosome Abnormalities," in Biological Effects of Neutron and Proton Irradiations, Vol. II, IAEC (Vienna, 1964), pp. 259-260.

23. H. J. Evans, op. cit., p. 254.

24. H. H. Smith, op. cit., p. 243.

25. H. J. Evans, op. cit., p. 260.

26. P. C. Gooch, et al., op. cit., p. 340.

27. Ibid.

28. H. H. Smith, op. cit., p. 235.

29. Ibid., p. 234.

VII. ABERRATION KINETICS

Although the methods of analysis have changed somewhat, attempts to quantify damage on biological tissue produced by radiation are not new. The purpose of this section is to present a couple of prevalent techniques used in the analysis of radiation data. In general, there are two approaches to the problem of the kinetics of radiation damage. Discussed first is the method of curve fitting. This method attempts to derive a simple quadratic equation relating the dose of radiation and the yield of aberrations. The second method discussed involves a statistical probabilistic approach. Here the aberration yield is expressed in terms of experimental parameters such as the dose of radiation, the LET, and the nature of the target (the chromosome). The two methods come together to give the same form of quadratic expression. It should be noted that this section deals primarily with chromosome aberration and not necessarily with cell survival, although attempts have been made to relate the two quantitatively.

Many studies have focussed on a comparison of the chromosome aberrations and the applied dose of radiation. Two classes of mechanisms are exhibited in the curves representing the experimental data. The two classes result in two types of kinetics. First, exchanges appear to be caused by the passage of a single ionizing particle. This accounts for the linear component of the dose-response curve. Second, exchanges appear which seem to be a result of the interaction of the effects of two independent ionizing particles. This is thought to be responsible for the dose-squared component of the curve. In its general form this

type of model has been reasonably confirmed by time-dose studies. The section on the linear energy transfer of the various radiations and their effects predicted several relationships. The differences in shape of the dose-response curves tend to confirm the predictions: With densely ionizing radiations, single-particle events dominate the radiation response; with sparsely ionizing radiations, events based on the interaction of two or more particles seem to play the leading role. Hence, as is seen in the section on RBE, the relative effectiveness of different radiations cannot follow the same kinetics due to the existence of two classes of mechanisms.[1]

Radiation effects do not correspond strictly to one or the other kinetic forms; rather, most radiation effects can be best described by the sum of the dose and the dose-squared component. In fact, it is generally accepted that there is a linear component in the dose-response for low-LET radiation for any type of aberration, even for exchanges which are commonly described as "two-hit." The relation between aberration yield (Y) and dose (D) of a low-LET radiation has been expressed as:

$$Y = K + \alpha D + \beta D^2 ,$$

where K is the spontaneous-aberration frequency. For a high-LET radiation, it is usually found, although not always, that the equation of the form

$$Y = K + \alpha' D$$

applies, where α, β, and α' are constants.[2]

As mentioned earlier, different forms of radiations give different

forms of chromosome aberrations. This is a result both of their differences in ionization density and types of interactions that must take place in order for a particular type of aberration to occur. In general, the larger share of the exchanges produced by low-LET X- or gamma-rays delivered at high intensity are taken into account by the dose-squared term; that is, most are produced by two independent breakage events and are called "two-hit" aberrations. The same is thought to be true for the chromosome interstitial deletions. The isochromatid aberrations, although clearly the result of two breaks, are the result of two breaks very close together and are primarily accounted for by the linear term even with X- or gamma-rays; apparently most are produced by "single-hits" or single-ionizing particles.[3]

Although it is still true that the aberration rate varies with the particular type of chromosome aberration being scored, higher LET radiations usually give a linear result based on "one-hit" kinetics. For example, regressions of aberration yield per cell on dose for all the classes of aberration at any of the LET values for protons and alpha particles in the experiments of Neary and Savage appear to be compatible with a linear relation. However, they found that in their experiment the aberration yield increased less rapidly than the first power of the dose. This result was not unexpected for gaps and minute intrachanges owing to a fall in scoring efficiency at higher levels of damage.[4]

With neutrons, all aberration types, including the two-break aberrations, are usually found to increase linearly with dose. This has been shown in plant studies such as those done by Neary,[5] by Conger,

et al.,[6] using 1.3-, 2.5-, and 14.1-Mev neutrons on Tradescantia microspores, by Giles[7] using Be,D neutrons, also on Tradescantia microspores, and also by Wolff, et al.,[8] for Vicia root tips irradiated with D,T neutrons. Surprisingly enough, however, Gooch found two-hit aberrations induced by 14.1-Mev neutrons. Although he found that the linear model could not be rejected on the basis of his experiments, the dose-square model gave him a much better fit to his data. In fact, Gooch found that his experiments with D,T neutron irradiation of human leukocytes suggested that in this system the kinetics for two-hit aberration production are nonlinear; they are very similar to the roughly dose-square kinetics usually observed with X-rays.[9] Nevertheless, Gooch found that his experiments with 2.5-Mev D,D neutrons, although incomplete, suggested that lower energy protons produced by the neutrons may have a high enough linear energy transfer to produce linear kinetics for two-hit aberrations in human leukocytes. Gooch further feels that if his other experiments support the dose-square kinetics for 14.1-Mev neutrons, it will be possible to estimate the radius of the sphere containing the volume within which breaks can interact in the leukocyte system.[10] Although Gooch's results were given in 1963, further substantiation has not appeared since.

Before moving on to the statistical approach to chromosome-aberration kinetics, a few sidelights implicated by the above results are in order. First, it is known that chromosome exchanges induced following thermal neutron irradiation increase linearly and not as the square of the dose. Since the neutron usually imparts its energy to some other

form of radiation as it passes through the biological matter, it can be implied that the aberrations are induced primarily by particulate radiation released following neutron capture. Furthermore, as will be discussed in the section on the relation of damage to oxygen content of the cell, the effect of such densely ionizing radiation following neutron capture is almost independent of the intracellular oxygen content, whereas the frequency of X-ray-induced aberrations is greatly influenced by oxygen tension.[11]

Secondly, it should be recalled that the aberration rate is not constant for any particular cell. There have been many reports that the sensitivity of cells to radiation changes throughout the cell cycle. Bacteria, for example, which have completed a cycle of DNA synthesis during which protein synthesis has been inhibited show an enhanced resistance to uv irradiation and to X-irradiation. Although it has not been totally confirmed (see Section I) this suggests that DNA might exist in a different physical state before and during replication.[12]

Thirdly, as is noted in the section on relative biological effectiveness, the comparison of the relative sensitivity of the cells to the different radiations is complicated by the existence of two kinetic forms: a curvilinear response of the two-break aberrations with dose of X- or gamma-rays and a linear response to dose of neutrons. Two aberration types, chromosome deletions and chromosome terminal deletions, are found to increase linearly with dose for all radiations and should be strictly comparable among all radiations. Chromosome terminal deletions, however, are much less common than the other types and are

moderately difficult to observe critically. This leads to proportionally larger errors for their coefficients than for the other aberration types.[13]

The following part of this section on aberration kinetics will deal with the statistical approach to chromosome aberrations as proposed by G. J. Neary along with John R. K. Savage. The primary source for this part is Neary's paper on "Chromosome Aberrations and the Theory of RBE;"[14] other sources than this primary source will be footnoted whenever employed. The outline for this part is as follows: first, the assumptions for the theory will be presented and discussed briefly; second, the classic form of Neary's result will be presented; third, approximations to the most general form will be presented and the result's similarities and dissimilarities to previous theories will be discussed; finally, more recent attempts at quantification of the parameters will be presented.

In general, for the simplest picture, Neary considers a site for aberration formation to contain two lengths of either the same or different chromosome. These constitute targets for the production of primary lesions. With this idea in mind, he makes six general assumptions, as follows:

(1) The chromosome is assumed to be a cylindrical filament, much along the lines of the Taylor model presented in Section I.

(2) The density of the target material is unity.

(3) The distribution of numbers of energy-loss events in a given track is Poissonian.

(4) The distribution of numbers of tracks through a target is Poissonian.

(5) Energy-loss events from the same track and from different tracks in a target act independently.

(6) Inherent intracellular recovery and repair processes are not explicitly specified in the model because the analysis presented is confined to single doses given in a short period of time.

The assumption dealing with the structure of the chromosome should be especially noted. It has been shown in Section I that this chromosome model is still much in doubt. Although it is possible that this assumption is true for the chromosomes of Tradescantia microspores on which most of the later experiments testing this hypothesis have been made, this does not mean that it is true for the chromosomes of all organisms. Perhaps a future research project could concern itself with the applicability of Neary's theory to a multi-stranded chromosome for longer dose times where recovery and repair are taken into account.

With the above assumptions as his base, Neary has derived the expression for the mean yield of aberrations per nucleus, Y, to be as follows:

$$Y = NE\{1 - \exp(-mgk^2)[1 - \{1 - \exp(-mk + mgk^2\}^2]\} ,$$

where N is the mean number of sites in a nucleus for a given type of aberration; E is the probability for interaction of primary lesions; m is the mean number of tracks through the mean projected area A of the chromosome segment of length l within a site; g is the probability that a track which has traversed one target chromosome of a site will also traverse the other; and k is the probability that passage of one

track through a target produces a primary lesion.

When several components of radiation are included each with its own value of m and k, the corresponding relation is as follows:

$$Y = NE\{1 - \exp(\Sigma\ -mgk^2)[1 - \{1 - \exp(\Sigma\ -mk + mgk^2)\}^2]\} \ .$$

By taking the lowest terms of the expansion of the exponentials, with $mk \ll 1$, or in other terms, $AD[1 - \exp(-p16Lt)]/16L \ll 1$ (where D is the dose absorbed in rads and L is the LET of tracks in kev/micron in unit density material, t is the diameter of the chromsome), or more simply, $Adt \ll 1$ ($At \approx 1.13 \times 10^{-3}$ microns^{-3} assuming unit density and $D \ll 900$ rads for Tradescantia microspores), the following relationship holds:

$$Y \cong NE\{mgk^2 + m^2k^2[(1 - gk)^2 - g^2k^2/2]\} \ .$$

In order to get this into a more familiar form, it should be noted that m, the mean number of tracks through area A, is thought to be related to the dose in the following manner: $m = 62.4AD/100L = AD/16L$. Now the familiar result previously mentioned in the first part of this section can be derived. The yield of aberrations at not too high doses can be seen to be the sum of a one-track term proportional to dose and a two-track term proportional to $(\text{dose})^2$. The two components of the yield may then be separated and called Y_1 and Y_2 respectively. With this substitution and the approximation that $p16Lt \ll 1$,

$$Y_2 = NE(pAtD)^2 \ .$$

Here the dose-squared part of the yield is shown to vary little with LET. With the same sort of approximation as above,

$$Y_1 = NEg(pAtD)(p16Lt) \ .$$

Here the linear part of the yield is directly proportional to LET, where p is the probability that a single energy-loss event in a target chromosome produces a primary lesion. Physically, in the case of linear dynamics, the proportionality to LET is thought to be the consequence of the production of two primary lesions by the same track; production of a single primary lesion (or two primary lesions by independent tracks) is approximately independent of LET. The general expression shows that, as LET is increased, the increase in Y_1 begins to fall below strict proportionality; a maximum is reached at an LET equal to 1.256/p16t followed by a decline. For example, if p = 1 and t, the diameter of the chromosome, is 2×10^{-3} microns, L_{max} = 39.2 kev/micron; for smaller values of p or t, the value of L_{max} would be higher. These calculations should bring to mind the discussion on the LET for the maximum damage discussed in the section on the relative biological effect.

The ratio of the yields is $Y_1/Y_2 = g16L/AD$. Since $g \sim t/\pi h$ and $A \sim ht$, where h is the radius of the site, $Y_1/Y_2 = 16L/\pi h^2 D$. This ratio is an interesting quantity to consider. The expression indicates the ratio is independent of the diameter of the chromosome and is determined solely by the LET, the dose and the site radius h. It should also be noticed that, at sufficiently small doses, the term Y_1 is greater than Y_2, whatever the value of the LET; but the ratio decreases as the dose is

increased. It is also seen from the expression that the magnitude of the dose at which the two yields are equal is greater the higher the LET.

The next problem to consider after the general formulation of this aberration-yield expression, is the quantification of the parameters and probabilities. It is necessary to see how these parameters may change from cell to cell and under the influence of modifying agents. For example, the parameter p, the probability that a single energy-loss event in a target chromosome produces a primary lesion might be thought to change due to certain chemical agents or environmental conditions. Also, E, the probability of interaction of two targets in which primary lesions have been formed might vary with the experimental conditions. The quantity E in turn could be influenced by recovery processes such as repair and thus would be susceptible to post-irradiation modification. The effects of post-irradiation handling was discussed in Section III. In order for there to be a modification of p, the agent would have to be present at the instant of energy absorption. Agents which may modify p and E might be the oxygen concentration in the cell (discussed in Section VIII) and sulphldryl compounds which tend to protect the chromosome. The magnitude of parameter changes is not known; however, experimentation with regard to oxygen enhancement ratio tells some of the story. Although this is discussed elsewhere, it should be noted here that they are dependent upon the LET of the radiation. p and E are not the only parameters which may be modified by a change in the environment or application of chemical agents. The target thickness t, or the site radius h, or the

number of sites, N, might all be changed, say with the stage in the cell cycle. However, these modifying mechanisms would not depend intrinsically on the LET of the radiation.

More problems arise when it is realized that secondary particles are emitted from the capture point of ionization site. These may have different LET's and the LET may vary appreciably over distance. For example, experiments with soft X-rays have shown that g (the probability that a track which has traversed one target chromosome of a site will also traverse the other) falls off when the track length of a low-energy electron is less than the site radius h. Also, delta-rays cause complications in the theory because the LET of a delta-ray changes considerably over a distance comparable to the site radius h. A given delta-ray which has crossed one target may not reach another. In order to meet this problem, "one-track" must be taken to mean that one primary track is involved but four possibilities must be considered: (1) the lesions in both targets are due to energy-loss events produced in them by the primary track; (2) the lesion in one target is due to the primary track and the lesion in the other is due to a delta-ray from this primary; (3) the two lesions are due to two separate delta-rays from the same primary; and (4) both lesions are produced by a single delta-ray.

Another problem exists in the definition and a quantification of the number of sites in the nucleus. In his paper, Neary considers a site as a region of radius h containing two separate sections of chromosome thread, each of mean length $4h/3$. If both sections are damaged by radiation, an aberration may result. If the two sections belong to different

chromosomes, this is a site for interchange; if both sections belong to two sister chromatids, an isochromatid site. The problem with this type of definition is that different types of interactions may have different distances over which the lesions may interact. For example, it has been found that there seems to be a qualitative difference between centric ring and dicentric sites; the factor which might account for the qualitative difference between sites is the existence of a different rejoining distance. It would be expected that the greater the distance over which exchange can take place, the larger the number of possible sites within the nucleus.[15] According to Savage in a later article, on the basis of pure random assortment of chromosome areas, a centric ring/dicentric ratio of 1:10 would be expected. However, at ana-telophase, the six median centromeres of the Tradescantia are found to move to the poles first, with subsequent packing of the 12 trailing arms parallel to one another. If this arrangement is maintained throughout interphase, Savage expects that complete interaction of breaks in the various arms would be limited by confining the possibility of exchanges to those arms in the immediate vicinity of one another. If it is then assumed that exchange is equally likely between breaks in any arms in fairly close approximity, Savage derives from possible arrangements of ana-telophase chromosomes a theoretical centric ring/dicentric ratio of 1:3. The observed ratio is 2.9:1.[16]

A problem now arises in the theory as to which parameter should take into account this ratio of aberrations. One possible solution would be to assume that the available number of sites of centric ring

and dicentric is also in the ratio of their observed aberration ratio. If proximity is the main condition determining the existence of a site, one would postulate that exchange to form a ring can take place over a larger distance than exchange to form a dicentric. However, this would mean that the probability of forming a ring is greater than that of forming a dicentric, a conclusion contrary to the aberration ratio found experimentally.[17] One could postulate that the average number of sites for the two aberrations is equal for a given dose. This would mean that the probability of exchange to form a ring in a ring site is less than that to form a dicentric in a dicentric site. However, this would be also contrary to the observed yield ratios. As of recently, the problem of assignment of the ratio factor to either p, h, or n has not been resolved.

The fact that Neary's theory for aberration yields includes so many parameters precludes the problem that the parameters are not always independent. The combination of experimental observations and postulation of the value of one parameter may lead to the evaluation of another parameter. The result may not seem realistic. Such a problem involves the evaluations of h (the rejoining distance) and N (the number of sites). Various attempts have been made to calculate the maximum distance over which rejoining may take place, that is, h. The original calculations gave the rejoining distance as 1 micron. More recently, Wolff, Atwood, Randolph, and Luippold calculated the distance to be closer to 0.1 to 0.3 microns. However, if these calculated distances are coupled with the evidence from exchange formation by very soft X-rays, then it is

calculated to be necessary to have hundreds of sites in any one nucleus.[18] This is in contradiction to calculations made by Atwood, who postulated that the value of n was 4 in Tradescantia, and by Savage, who postulated that the average value of n in Tradescantia is 2.4.[19] Although all these calculations have led the researchers in this area into a bind of contradictions, it is still believed that these parameters represent some characteristics of the cell, that is, real physical entities. This type of analysis continues.

One way of testing this theory is to compare the calculated values of some of the parameters with the values found in micrographs of the cell chromosomes. This also serves to test various postulates for the structure of the chromosome. For example, calculations of the value of pt, or the effective thickness of the target, have been found to equal approximately 4.8 to 5 A with a corresponding estimated LET of 165 kev/micron in Tradescantia. This target thickness of about 5 A is thought to be suggestive of an actual target consisting of a basic DNA-protein thread or simply a DNA double helix or even one of the helices.[20]

Although there are many problems to be resolved in this sort of statistical approach, the method has several distinct advantages. First, the model offers a complete basis for RBE for chromosome aberrations, that is, a comparison of the yields for various experimental conditions. This model gives a relationship of RBE with both dose and LET of the radiation, as well as including parameters for the particular characteristics of the nucleus. Secondly, the model can be adapted to show a

relationship between aberration formation and cell killing. Although the model would not imply a direct causal connection between aberrations and killing, it would show a kinetic similarity if there were double-target sites for cell killing analogous to but not necessarily identical with aberration sites. Such a killing site in a diploid cell might be a place where two homologous loci on homologous chromosomes were in close relationship. There could be a one-track and a two-track process for killing the site, that is, producing recessive lethal damage in both loci. The same formal dependence on dose and LET for the one-track and two-track processes present in Neary's theory would then follow. Neary extends his model to this killing model in the reference paper sited in the beginning of this part.

In conclusion, it should be remembered that the statistical approach, as proposed by Neary in one form and by Lea in another form in 1955, is not in basic disagreement with the basic model of the yield varying with the dose and the square of the dose; rather, the statistical approach is designed to include the parameters not included in the macroscopic theory. If the contradictions in the statistical approach can be worked out, and if the statistical approach can be modified to include the possibility of multi-strandedness of the chromosomes and repair and recovery, it will prove to be a very useful theoretical model as it includes the radiation condition, oxygen concentration, dose, and LET, as well as characteristics of the particular cell being irradiated.

FOOTNOTES FOR SECTION VII

1. H. H. Smith, J. L. Bateman, H. Quastler, and H. H. Rossi, "RBE of Monoenergetic Fast Neutrons: Cytogenetic Effects in Maize," in Biological Effects of Neutron and Proton Irradiations, Vol. II, IAEC (Vienna, 1964), p. 234.

2. G. J. Neary, J. R. K. Savage, H. J. Evans, and J. Whittle, "Ultimate Maximum Values of the RBE of Fast Neutrons and Gamma Rays for Chromosome Aberrations," in International Journal of Radiation Biology, 6, No. 2, 127 (1963).

3. Alan D. Conger, M. L. Randolph, C. W. Sheppard, and Helen J. Luippold, "Quantitative Relation of RBE in Tradescantia and Average LET of Gamma-Rays, X-Rays, and 1.2, 2.5, and 14.1 Mev Fast Neutrons," in Radiation Research, 9, 533 (1958).

4. G. J. Neary and J. R. K. Savage, "Chromosome Aberrations and the Theory of RBE: II. Evidence from Track-Segment Experiments with Protons and Alpha Particles," in International Journal of Radiation Biology, 11, No. 3, 211 (1966).

5. H. J. Curtis, J. Tilley, and C. Crowley, "The Cellular Differences between Acute and Chronic Neutron and Gamma-Ray Irradiation in Mice," in Biological Effects of Neutron and Proton Irradiations, Vol. II, IAEC (Vienne, 1964), p. 151.

6. Conger, et al., op. cit., p. 533.

7. P. C. Gooch, M. A. Bender, and M. L. Randolph, "Chromosome Aberrations Induced in Human Somatic Cells by Neutrons," in Biological Effects of Neutrons and Proton Irradiations, Vol. I, IAEC (Vienna, 1963), p. 340.

8. Ibid.

9. Ibid.

10. Ibid.

11. H. J. Evans, "Chromosome Aberrations Induced by Ionizing Radiations," in International Review of Cytology, Eds. G. H. Bourne and J. F. Danielli, 13, 260 (1962).

12. K. V. Shooter, "The Effects of Radiations on DNA Biosynthesis and Related Processes," in Progress in Biophysics and Molecular Biology, Eds. J. A. V. Butler and H. E. Huxley (Pergamon Press, 1967), p. 304.

13. Conger, et al., op. cit., p. 536.

14. G. J. Neary, "Chromosome Aberrations and the Theory of REB," in International Journal of Radiation Biology, 6, (1963).

15. J. R. K. Savage, "Chromosome-Exchange Sites in Tradescantia-Paludosa Microspores," in International Journal of Radiation Biology, 9, No. 1, 88 (June 10, 1965).

16. Ibid., p. 90.

17. Ibid., p. 93.

18. Ibid., p. 84.

19. Ibid., p. 84.

20. Neary and Savage, op. cit., p. 216.

VIII. OXYGEN CONSIDERATIONS

One of the problems with the earlier work on chromosome aberrations and the comparison of effects of different forms of radiation was that the experimenters were unaware of the influence of oxygen on aberration frequencies. Although it has been shown that oxygen conditions have little effect during high-LET irradiation, the conditions do have an effect on sparsely ionizing radiation. This has a significant effect on the evaluated RBE's. As a result, certain of the quantitative conclusions which were arrived at early in the abberation research are found not to be directly applicable. A case in point is irradiation carried out under anoxic conditions.[1] This section is designed to explain the effects of oxygen on an aberration experiment. The statistical implications will also be discussed.

The so-called oxygen effect was mentioned earlier in Section II. It was explained that the yield of hydrogen peroxide, one of the agents inducing aberrations indirectly, is found to be dependent on the presence of oxygen in the case of X-irradiated water. However, it was also explained that this effect plays a smaller role in the formation of aberrations by neutrons and alpha particles where H_2O_2 is produced independently of the presence of oxygen. This is a result of the fact that with densely ionizing radiation, the close spacing of the OH radicals leads to the formation of H_2O_2 whether or not free oxygen is available.

Oxygen conditions have been found to play no small role in the determination of the RBE. For example, in the work of Conger on the

RBE of fast neutrons to X-rays for inducing abnormal anaphases in Ehrlich ascites tumor cells, it was found that the efficiency of neutrons compared to X-rays in these experiments was found to be 2.5:1 in oxygen, but 6:1 under anoxic or nitorgen conditions.[2] Comparable results have been found by Hornsly, et al., in a comparison between fast neutrons (D → Be) and 1.5-Mev X-rays under different conditions of oxygenation.[3] Similarly, in plants, the relative efficiencies of fast neutrons (D → ^{6}Li D) and gamma-rays (Co^{60}) in inducing micronuclei in Vicia Faba root-tip cells was found to be 10.5:1 if both irradiations were given in air; a value of 18:1 would be expected if the irradiations were performed in the absence of oxygen.[4] It is interesting to note the effects of oxygen conditions on the formation of aberrations was first found in the comparison between alpha-radiation and X-irradiation in both Vicia and Tradescantia. It was found that the effect of alpha-radiation was almost independent of oxygen concentration. From this result, it was postulated that fast neutrons should yield an oxygen factor somewhat intermediate between X-rays and alpha-particles. Later it was found by Giles, et al., that this was the case; the ratio of the doses of fast neutrons given in nitrogen and in air, in order to produce approximately equal aberration yields was about 1.4:1.[5]

One of the advantages of the statistical model described in Section VII is that the model can take into account the change in the conditions of the experiment, such as the change in oxygen condition. In the statistical model, the oxygen condition is taken into consideration by the variation of two parameters, p and n. Oxygen is postulated to act

as a modifying agent and thus is thought to affect the parameter p (the probability that a single energy-loss event in a target chromosome produced a primary lesion).[6] The oxygen conditions are also thought to affect n (the mean number of sites in a nucleus for a given type of aberration). Although there is believed to be no significant difference in the values of n for nitrogen or oxygen conditions, there is a suggestive pattern indicating a slight increase in average site number if air or oxygen is present at the time of irradiation. This trend is found to be slightly more marked for centric rings. In general, their mean site number is found to be very slightly larger than for dicentrics. This effect might be observed as a change in the ratio of yields of dicentrics to centric rings for radiation in air and nitrogen. Here a greater increase in centric ring sites in air would lead to a larger value for this ratio. In fact, the regression coefficients (dicentrics on centric rings) are in air 2.75 ± 0.22 and in nitrogen 3.13 ± 0.24.[7]

One of the ways to account for the variations in oxygen conditions is to take into account the effect of a change of p on another parameter k (the probability that passage of one track through a target produces a primary lesion). With the necessary assumptions, Neary has derived the following expression: $k = [1 - \exp(-pLt)]$, where L is the LET and t is the diameter of the chromosome. The ratio of the values of the quantity k in the presence and absence of oxygen at a given LET can be denoted λ. Originally, the quantity that was used for comparison of gas conditions was the Oxygen Enhancement Ratio or OER. It is defined as the ratio of doses in the two gas conditions required to form the same number of

aberrations. One of the problems with this quantity is that the OER derived from a two-track process is not directly comparable with that for the one-track process at the same LET. The advantage of the quantity λ is that it can take these differences into account; that is, although the OER for the two-track process can be seen to be equal to λ, the OER for the one-track process of aberration is equal to λ^2.[8]

Using this notation, the oxygen factor for both processes can be combined to form an oxygen factor for the total aberrations. For the first component of single gaps (single-lesion gaps) the yield without oxygen would be $1/\lambda$ of the yield with oxygen. For the second component (double-lesion gaps), the ratio would be $(1/\lambda)^2$, where λ is the oxygen factor for primary lesions deduced from the aberration data. Since the yield of both types of single gap is proportional to dose, the over-all dose-modification factor for single gaps is equal to the ratio of total yields with and without oxygen. If the two components of single-gap yield per unit dose in oxygen are denoted by C_1 and C_2, the over-all oxygen factor is found to be:

$$\frac{C_1 + C_2}{C_1/\lambda + C_2/\lambda^2} = \frac{\lambda^2(C_1 + C_2)}{\lambda C_1 + C_2},$$

where C_1 and C_2 are obtained from the yield of gaps as a function of LET and λ^2 is the OER for aberrations from OER as a function of LET.[9] Work is now being done to quantify these results.

The effect of LET on OER should be noted in this analysis. In general, the general qualitative pattern for radiobiological effects

suggests there is a fall in the OER with increasing LET. It has been explained that a physico-chemical process independent of the essential aberration process is mainly responsible for the detailed features of the variation with LET. This trend is taken into consideration through the parameter k. As can be seen in the equation for k, as the LET increases toward higher values, the value of k increases toward one irrespective of whether oxygen is present or not.[10] This sort of reasoning has proven to give reasonable results in the experiments thus far performed.

FOOTNOTES FOR SECTION VIII

1. H. J. Evans, "Chromosome Aberrations Induced by Ionizing Radiations," in International Review of Cytology, Eds. G. H. Bourne and J. F. Danielli, 13, 269 (1962).

2. Alan D. Conger, Radiology, 66, 63 (1956).

3. Hornsey, et al., International Journal of Radiation Biology, 2, 37 (1960).

4. H. J. Evans, op. cit., p. 272.

5. Ibid., p. 275.

6. G. J. Neary, "Chromosome Aberrations and the Theory of RBE," in International Journal of Radiation Biology, 6 (1963).

7. J. R. K. Savage, "Chromosome-Exchange Sites in Tradescantia-Paludosa Microspores," in International Journal of Radiation Biology, 9, No. 1 89 (June 10, 1965).

8. G. J. Neary and J. R. K. Savage, "Chromosome Aberrations and the Theory of RBE" II. Evidence from Track-Segment Experiments with Protons and Alpha Particles," in International Journal of Radiation Biology, 11, No. 3, 220 (1966).

9. Ibid., p. 221.

10. Ibid.

CONCLUSION

It is difficult to write a conclusion for a paper of this sort because little in the field has been conclusively proven. The majority of the ideas presented in the paper have yet to be accepted by a majority of researchers and many of the ideas are destined for replacement. Nevertheless, aberration studies are continuing at a fast pace and the postulations presented in this paper may be the so-called facts of the future.

Section I introduced two conflicting models for chromosome structure. The first model postulated the existence of a single-stranded chromosome that is coiled, looped, and folded into the chromosome body. The elementary chromosome fibril is thought to have a diameter of 100 A, consisting of two 30 to 40 A DNA-histone molecules arranged side by side. The cross-linking is postulated to be accomplished by histones or other proteins. The alternate model suggests that the chromosome is composed of several of these basic fibrils. Looping, folding, and coiling also has a place in the second model. The chromosome, in the second model, is thought to have a diameter ranging from 400 A on up. Recent evidence has suggested that both models have distinct probability of being the correct model. However, it is safe to say that the chromosome structure is dependent on the organism involved. It seems likely that lower organisms most likely conform to the former model. However, it also seems likely that higher organisms have multi-stranded chromosomes. The resolution of chromosome structure is just one problem that remains to be investigated.

Section II discussed the biochemical aspects of radiation damage. It was shown that radiation can produce indirect damage on the chromosome by creating radiolysis products of water. These products can then attack the chromosome at several levels of structure. The bases of the DNA seem to be the main targets for radical action with the purines being more resistant to radiation-induced degradation than the pyrimidines. The sugars and the phosphate linkages are also open to radical attack, although naturally occurring components of the cell can lessen the effects. Radiation can also produce direct chromosome damage by producing ionizations near the chromosome threads. This effect becomes more important as the concentration of DNA is increased in the cell. Previous evidence indicated that several ionizations were needed to produce damage in the chromosome; however, more recent studies have shown that a single ionization can create enough damage for a possible aberration site. Also, another direct effect of radiation is the breakage of hydrogen bonds in the DNA. A visible product resulting from chromosome damage is the clumping of the chromosomes.

Section III was devoted to a discussion of chromosome repair. It was found that the cell is capable of repairing damage produced by gamma- and X-rays, but it is not capable of repairing damage produced by more densely ionizing radiations. The reason for this is not known positively; however, it is thought that since neutrons are so densely ionizing that if a chromosome is hit at all, two or more closely spaced ionizations would most likely lead to permanent damage. The lack of recovery following neutron irradiation is not always observed due to

the ability of some cells to bypass chromosome damage. Although it is known that repair does occur, the repair mechanism is not fully understood. Neither is it known how long breaks remain open. It is thought, however, that the time that breaks remain open is dose-dependent, and repair is more efficient the longer the time before DNA replication begins.

Section IV discussed the formation of aberrations. Here again the mechanism is not well understood. Two models exist in the literature. One model postulates that radiation serves to break the chromosome threads which are then open to exchange with other closely spaced and closely timed chromosome breaks. This is called the breakage-and-exchange model. The alternative model postulates that the radiation serves to damage the chromosome in such a way that proximate chromosomes can exchange parts. In this second model, the primary event is the ionization action creating a temporary possible site for exchange rather than a break which needs to be repaired. The temporary damage will decay unless there is another damaged point available within a short time and distance to react with it. The second model was created to explain discrepancies and unexplained evidence in the first model. Although this model has yet to be generally accepted, recent evidence tends to support its hypothesis. However, even the second model cannot explain some of the evidence found in experiment. The fourth section also described the various aberration classification schemes used by researchers today. It was shown that the designation of aberration type is dependent on the component of the chromosome involved, the

completeness of the exchange, and the observed aberration configurations. The dependence of the type of aberration produced on the stage of the cell cycle was also discussed. It was shown that the aberration type is very dependent of the state of the chromosome during irradiation.

Section V introduced the concept of linear energy transfer, or the number of energy-loss events per length of the partical track. The LET was shown to define the quality of the radiation particle. The higher the LET, the more densely ionizing is the radiation. This creates more ionizations per length and thus is capable of creating damage points in the chromosome much closer together. As a result, it was pointed out that many biological systems are much more sensitive to damage by neutrons than by X- or gamma-rays. For example, the aberration production per unit dose increases with LET. Similarly, cell killing increases with LET. It was also pointed out that the important consideration was the LET of the radiation particle rather than the nature of the radiation particle itself.

Section VI presented a discussion of the relative biological efficiency (RBE) in the consideration of aberration production. The RBE was found to depend on several parameters, including total dosage, dose rate, time until inspection, LET, particle type, types of biological damage observed, stage of cell development, volume of the chromosome, and type of tissue inspected. No single value for RBE was stated for this reason. The RBE was defined as the ratio of the energy imparted to a unit mass by therapy X-rays to the energy imparted to a unit mass by the given

type of radiation in order to produce the same effect. For neutrons compared to X-rays, it was found that the RBE varied from a value around 2 for 14.1-Mev neutrons, to a value of 5 for fission-spectrum neutrons, to a value approaching 100. One of the problems in determining the RBE is that dose-response curves differ for radiations of different LET and dose rate. For this reason, a value for the RBE is meaningless without the dose or dose-rate imposed in the experiment.

Section VII presented two kinetic models for aberration production. The first model suggested that the yield of aberrations was a function of the first and second powers of the dose, that is, $Y = k + aD + bD^2$, where k, a, and b are constants determined by a fit of experimental curves. Although this formulation can be imposed upon a curve, it does not tell very much about the characteristics of the chromosome and of the radiation particle involved. The second model presented was a statistical derivation of aberration yields as a function of many of the experimental parameters and probabilities. The value of this approach is that it takes the particular experimental conditions into consideration. In this way, all the dimensions of the chromosomes and possible interaction distances are included, as well as particular information about the particle such as its LET and the dose imposed. However, there are so many unresolved parameters that this method also resorts to curve fitting in the end. Also this model postulates the existence of certain parameters which may not physically exist. For example, one of the parameters is the number of sites for chromosomal damage, and such things may not exist, as recent evidence seems to

indicate. Another problem with the statistical model is that it assumes a single-stranded chromosome, which may not be the case for all organisms. Also, the repair or recovery ability of the cells is not taken into consideration. However, there is one redeeming factor: after simplifications, the two models boil down to the same functional relationship.

Section VIII considered the effects of the oxygen environment during the course of the experiment. It was pointed out that oxygen conditions play a more influential role for the less dense ionizing radiations than for the more densely ionizing radiations. The reason for this is that densely ionizing radiations produce OH radicals so close to each other than H_2O_2, a molecule which attacks the chromosome, is formed whether or not free oxygen is available. This is not true for the less densely ionizing radiations such as X-rays and gamma-rays. Thus the evaluation of RBE is dependent on the oxygen conditions of the experiment. The kinetics of the oxygen factor is also considered and is found to be dependent on the LET as expected.

As can be seen, the state of the art of chromosome-aberration research is still in flux. Much needs to be determined. An interesting point is each bit of information in one area of the problem influences all the other areas. But, as each bit of information is established, the whole area of research comes closer to a realization of its goal.

GLOSSARY

Acentric. Chromosome fragment lacking a centromere.

Achromatic lesion. A section in the chromosome picking up no stain.

Allium. A genus of bulbous herbs distinguished by the characteristic odor, sheathing, mostly basis leaves, and umbellate white, yellow, or red flowers.

Anaphase. The stage in mitosis in which the chromosome halves move toward the poles of the spindles.

Bacterial viruses (Bacteriophage). Viruses that multiply in bacteria.

Base analogs. Purines and pyrimidines which differ slightly in structure from the normal nitrogenous bases.

Centric ring. Chromosome fragment ring having a centromere.

Chiasma (pl. chiasmata). A fusion and exchange of segments of chromatids occurring between members of a bivalent during diplotene.

Chromatid. A daughter strand of a duplicated chromosome which is still joined to the other daughter to a single centromere.

Chromatid aberration. Aberrations in which the unit of aberration formation is the chromatid.

Chromatin. The granular protoplasmic substance in the nucleus of the cell that readily takes a deep stain. The chromosomes are included in the chromatin.

Chromomere. Bead-like areas of increased density along the chromonemata.

Chromonema (pl. chromonemata). The fundamental element of chromosomes which are observed in the light microscope as threads.

Chromosome-type aberration. Aberrations which involve both chromatids of a chromosome at identical loci.

Chromosin. An acid protein rich in tryptophan regarded as a major component of the chromosomes.

Deletion. Loss of a section of the genetic material from a chromosome.

Delta-rays. Secondary radiation, composed of electrons, produced by primary radiations.

Deoxyribonucleoside. The condensation product of a purine or pyrimidine with the five-carbon sugar, 2-deoxyribose.

Deoxyribonucleic acid (DNA). A polymer of deoxyribonucleotides.

Deoxyribonucleotide. A compound which consists of a purine or pyrimidine base bonded to the sugar, 2-deoxyribose, which in turn is bound to a phosphate group.

Dicentric. Chromosome thread having two centromeres.

Diploid. The state of the chromosome in which each type of chromosome except for the sex chromosome is always represented twice.

Diplotene. The stage of the meiotic prophase immediately following pachytene during which the homologous chromosomes tend to repel one another.

Escherichia Coli. A genus of aerobic rod-shaped bacteria.

Histone. Basic protein molecules having a net positive charge.

Interchange. Aberrations with exchanges between chromosomes.

Interphase. The period between any two mitoses of a nucleus.

Interstitial deletion. Small fragments deleted from between two

breaks very close to one another in the same chromosome.

Intrachange. Aberrations with exchanges occurring within the chromosome.

Isochromatid break. Aberration where sister chromatids are broken in practically identical loci in both chromatids.

Isodiametric deletions. Small bodies, presumed to be chromosome rings, most commonly of about 1 micron diameter.

Lampbrush chromosome. A greatly enlarged pachytene chromosome having apparently filamentous granular loops extending from the chromomeres.

Lilium. A large genus of herbaceous plants having scaly bulbs, whorled or scattered leaves, showy flowers with a perianth of six segments, versatile anthers, a 3-lobed stigma, and a capsular fruit.

Linear energy transfer (LET). The number of energy-loss events per unit length of the track of an ionizing particle in units of kev/micron.

Nucleohistone. The combination of nucleic acids and histones.

Oxygen enhancement ratio. The ratio of doses in two gas conditions required to form the same number of aberrations.

Pachytene. A stage of the meiotic prophase that immediately follows zygotene and is characterized by the splitting of paired chromosomes into chromatids.

Polyteny. The state of having many units of reduplicated chromonemata in close longitudinal association.

Protease. An enzyme which hydrolyzes proteins and peptides.

Rad. The quantity of radiation which results in the absorption of

100 ergs of energy per gram of the irradiated material.

Recombination. New combinations of linked genes.

Relative biological effect. The ratio of the energy imparted to a unit mass by therapy X-rays to the energy imparted to a unit mass by the given type of radiation in order to produce the same effect.

Roentgen. The dose of radiation which produces 2.1×10^9 ion pairs in a volume of 1 cubic centimeter of air.

Saline. A salt-containing solution.

Thermal neutron. A neutron slowed down by collisions with light atoms to the speed of gas molecules at room temperature (about 0.025 ev at $15^{\circ}C$).

Thymine dimer. The linking of two thymines in adjacent nucleotides through the opening of double bonds.

Tradescantia. A genus of American herbs comprising the spiderworts and having mostly narrow elongated leaves and large white, pink, or violet ephemeral bracteate flowers.

Triturus Viridescens. One form of a newt.

Vicia. A widely distributed genus of often climbing herbs having pinnate leaves and blue, purple or yellow flowers.

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