RADIATION BIOLOGY

VOLUME I: HIGH ENERGY RADIATION

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PART II

CHAPTERS 9 TO 18

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CHAPTER 9

Chromosome Aberrations Induced in Animal Cells by Ionizing Radiations[1]

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Introduction. Nature of the induced rearrangements: Methods of diagnosis—Types of induced chromosomal aberrations. The process of structural rearrangement: The breakage process. Differences in sensitivity to ionizing radiations: Relative sensitivity of different organisms—Effect of ploidy—Relative sensitivity of chromosomes in different types of cells of the same species—Changes in sensitivity of chromosomes in cells of the same type. Chemical and cytochemical studies. References.

1. INTRODUCTION

The effects on living cells of higher plants and animals of exposure to ionizing radiations are evidenced by various alterations in the constitution and behavior of cellular materials. Among the most readily detectable reactions are those that modify the form and pattern of association of chromosomes and the course of their separation in the cycle of mitosis. Cytologic examination of irradiated cells has disclosed a variety of aberrant types, in which the chromosomes were either adherent or clumped, fragmented or reconstituted, excessive or deficient in number. Many of these abnormalities were observed in the pioneer studies of the biologic effects of radiation carried on during the early years of the century (for example, Bergonié and Tribondeau, 1904, 1906, on rat testes; Krause and Ziegler, 1906, on various mammalian tissues; Perthes, 1904, P. Hertwig, 1911, Payne, 1913, and Holthusen, 1921, on eggs of *Ascaris;* Koernicke, 1905, and Gager, 1908, on somatic and meiotic cells of plants; Mohr, 1919, on testes of grasshoppers; Amato, 1911, and Grasnick, 1918, on cells of amphibia). However, no consistent interpretation of the nature and sequence of origin of the various types of aberrations was developed. This was partly because most of the observations were made

[1] This manuscript was prepared prior to Apr. 1, 1951. Although a few changes have been made since then, this essentially represents the literature available at that time.

on sectioned material, in which induced breaks were not easily diagnosed. Moreover, adequate consideration was not given to the effect on the induced reaction of the length of time elapsing between irradiation and fixation of the cell.

Recognizing the need for a more extensive evaluation of these factors, Alberti and Politzer (1923, 1924) examined entire cells of the corneal epithelium of salamander larvae, fixed at varying periods of time after the animals had been exposed to X-ray treatments of different intensities. The observed types of cellular disturbance were interpreted as revealing a reaction system that involves (1) a period of "primary effect," beginning shortly after irradiation, characterized by a decline in the frequency of mitoses and the appearance, especially after treatment with high doses, of pycnotic nuclei with adherent chromosomes, (2) a subsequent period of mitotic inactivity, and (3) a period of "secondary effects," characterized by abnormal mitoses with fragmented or reconstituted chromosomes, whose frequency is dependent on the duration of the treatment.

It is now recognized that during the period of "primary" or "physiological" effect the materials of the chromosomes are altered, and the mitotic mechanism governing the normal orderly distribution of the chromatids into daughter nuclei is inhibited. In cells that have been exposed to moderate doses of radiation, the chromosomes in late prophase, metaphase, or anaphase stages may continue the course of division, but the mitotic progress of cells in earlier prophases is arrested, and at times their chromosomes evince regressive changes that suggest a return to interphasic conditions (see, for example, Carlson, 1940). Because the onset of new mitoses is inhibited, there occurs shortly after irradiation a period of mitotic inactivity, in which practically all cells appear to be in resting stages. If the treatment has not been too intense, the mitotic rhythm may subsequently be reestablished, whereupon the condensed chromosomes may reveal various types of induced abnormalities. At times the delay effected by radiation may be followed by precocious differentiation of the treated cells (e.g., primary spermatocytes into giant spermatids, Creighton and Evans, 1941).

When the treatment is more drastic, the chromonematic threads of early mitotic stages may reveal nodal thickening along their lengths, and the condensed chromosomes may adhere or clump to form irregular aggregates of chromatin material, presumably as a consequence of changes in viscosity of component proteins and nucleic acids. Such alteration disrupts the normal pattern of chromosome division, at times producing bizarre mitotic figures (see, for example, Helwig, 1933, White, 1937, Carlson, 1938a, Carothers, 1940, Creighton and Evans, 1941, and Bishop, 1942, on grasshopper cells; Bauer and LeCalvez, 1944, on eggs of *Ascaris;* Tansley, Gray, and Spear, 1948, Duryee, 1949, and Rugh, 1950, on amphibian cells; Welander *et al.*, 1948, on cells of embryos and larvae of

salmon; Lasnitzki, 1943b, 1948, on avian tissue cultures; Pfuhl and Küntz, 1939, on connective tissue cells of rabbits; Koller, 1947, on normal and malignant cells of man). A few selected illustrations are presented in Fig. 9-1. Alberti and Politzer realized that pycnotic chromosome masses represent one aspect of cellular necrosis. When alteration of the chromosomes is so complete that individual members of the set cannot be recognized, the aberrations are not serviceable for quantitative studies of breakage, but are useful, as will be shown presently, for chemical and cytochemical analysis of the changes effected in the nucleic acids and proteins that represent the major organic constituents of chromosomes.

Of greater use in quantitative studies are those "secondary" or "genetic" effects induced by moderate doses of radiation, in which

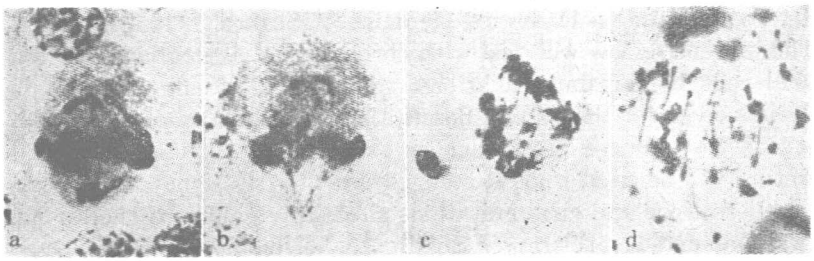

FIG. 9-1. Abnormal mitoses, with adherent chromosomes resulting from exposure of cells to ionizing radiations. (*a*) (*b*) From grasshopper neuroblasts (*Carlson*, 1941b); (*c*) (*d*) from malignant cells of man (*Koller*, 1947).

damage is sufficiently localized to break the chromosomes without impairing permanently the synthetic and reparative processes essential to mitotic and other vital cellular activities. Under these conditions the chromosomes may establish new associations by union of their broken ends. The resulting rearrangements can be detected by examination of the treated cells or their descendants, or by genetic analysis of individuals carrying the aberrations. Production of such viable chromosomal exchanges by X-ray treatment was first reported by Muller (1928a, b) and Muller and Altenburg (1928, 1930), who designed excellent methods for detection and preservation of the induced rearrangements. These furnished a wealth of experimental material, whose analysis during the second quarter of this century has greatly furthered understanding of the mechanisms of heredity and evolution. Determination of the frequency of these gross chromosomal aberrations under various experimental conditions has also furnished basic data for a preliminary analysis of the processes involved in chromosome fragmentation and reconstitution. The significance of such data in supplying information about the mode of action of ionizing radiations and the possible control of deleterious effects has become increasingly apparent in recent years.

Many aspects of such studies have been examined in extensive detail in a series of reviews, texts, and symposia since the publication of Duggar's "Biological Effects of Radiation" in 1936 [e.g., Timoféeff-Ressovsky, 1937; Timoféeff-Ressovsky and Zimmer, 1939; Bauer, 1939c; Delbrück, 1940; Muller, 1940, 1950b; Fano and Demerec, 1944; Catcheside, 1945, 1946, 1948; Lavedan, 1945; Gray, 1946; Spear, 1946; Lea, 1946; Giese, 1947; Buzatti-Traverso and Cavalli, 1948; Fano, Caspari, and Demerec, 1950; Sparrow, 1951; and the numerous contributions to the 1941 Cold Spring Harbor Symposium on Genes and Chromosomes, the 1946 London Conference on Certain Aspects of the Action of Radiations on Living Cells (published in 1947), the 1948 Brookhaven Conference on Biological Applications of Nuclear Physics, the FIAT Review of German Science from 1939 to 1946 (published in 1948), and the 1948 Oak Ridge Symposium on Radiation Genetics (published in 1950)].

The present review will deal with chromosomal aberrations induced in animal cells by ionizing radiations. Separation of animal from plant materials, even for the sake of description, imposes arbitrary limitations that are not always advantageous; accordingly, pertinent botanical literature will be cited when it seems desirable. Designation of chromosome aberrations and gene mutations as sharply delimited classes is also to a large extent arbitrary. Small chromosomal aberrations cannot always be distinguished from the so-called "point mutations." Moreover, radiation studies have clearly shown that the reaction system responsible for the characteristic phenotypic expression of a gene may be profoundly altered by a realignment of parts of chromosomes in the process of structural rearrangement. It is thus apparent that no sharp line can be drawn between gene mutations and chromosome aberrations; but this article will be limited to a consideration of the types of gross chromosomal alterations that can be detected either by direct cytological examination or by breeding tests. Even with attention focused on this restricted segment of a large body of information, it will not be possible to review all the available experimental evidence, and the discussion will be concerned primarily with the types of induced aberrations, the methods used in their diagnosis, and their significance with respect to evaluation of the processes of chromosome breakage and recombination. The effects of radiations in retarding mitosis and in modifying the normal distribution of chromosomes on the spindle are considered in Chap. 11 by Carlson.

2. NATURE OF THE INDUCED REARRANGEMENTS

Various types of cells from many different species of plants and animals have been irradiated in order to obtain information about the process of structural rearrangement, but the majority of available data have been

obtained in studies of the effects of treating microspores, or pollen grains, of plants of the genus *Tradescantia*, and spermatozoa, or male gametes, of flies of the genus *Drosophila*. Analysis has been facilitated by the relatively small number of chromosomes in these two types; in the species of *Tradescantia* most commonly used the haploid number is six, and in *D. melanogaster*, the most widely studied species of *Drosophila*, the hap-

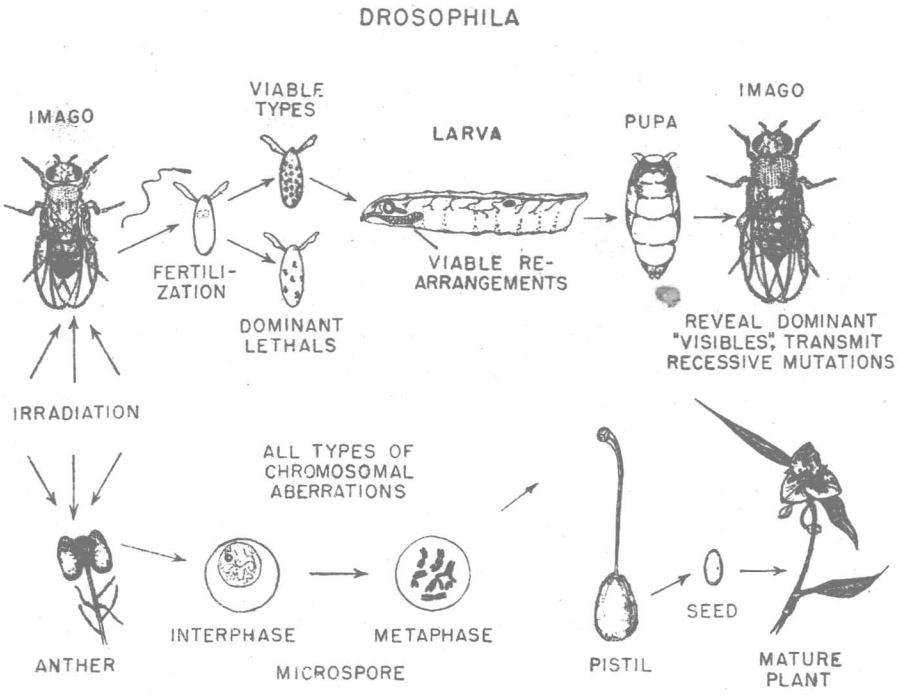

FIG. 9-2. Diagram of development stages in *Drosophila* and *Tradescantia*, indicating sources of materials for cytogenetic studies described in text. (*From Kaufmann*, 1948a.)

loid number is four (either X, 2, 3, 4 or Y, 2, 3, 4—the letters indicating the sex chromosomes, the numerals the autosomes). The techniques employed in studying these organisms illustrate the methods commonly used to detect chromosomal aberrations (Fig. 9-2).

2-1. METHODS OF DIAGNOSIS

In studies on *Tradescantia* the irradiated cells are themselves examined after an interval of time sufficient to permit the treated chromosomes to reach the condensed stages, when determination of the number of fragments or chromosome exchanges is feasible. The earlier studies, on both plant and animal cells, employed this method in basic form; but the

development of smear techniques, especially for spreading and staining the microspores of plants (Taylor, 1924; Kaufmann, 1927), presented the opportunity—used advantageously in the work on *Tradescantia*—of irradiating large numbers of cells in a known stage of microsporogenesis and obtaining, by subsequent inspection of the stained metaphases and anaphases, extensive and comprehensive data on the frequency of induction of fragments and various types of chromosomal rearrangements. Diagrams illustrating the kinds of aberrations detected in such studies of *Tradescantia* have been presented by Catcheside (1945, 1946, 1948); Catcheside, Lea, and Thoday (1946a); and Lea (1946).

Comparable although less extensive studies have also been made by direct examination of irradiated animal cells (for example, the studies of Carlson, 1938a, 1941b, on neuroblast chromosomes of the grasshopper, *Chortophaga*). The effects of the treatments are detectable as breaks or lesions along the chromosomes, or as new associations of the breakage ends (represented diagrammatically in Figs. 9-3 and 9-4). An interpretation of the mode of origin of the types of breaks designated in these diagrams as "chromosome," "chromatid," and "isochromatid" will be presented subsequently.

From these illustrations it is apparent that cytologic examination of condensed chromosomes at metaphase or anaphase will reveal all types of induced aberrations, including lethal as well as viable aberrations. Differentiation of the two classes is desirable in some types of analysis. It is also desirable to know more about the precise location of breaks and the complexity of individual rearrangements than can be inferred from observations of mitotic chromosomes. Studies on *Drosophila* have been especially useful in supplying such information.

In the method commonly used for detection of induced chromosomal rearrangements in *Drosophila*, gametes are irradiated by treatment of males, which are then mated with untreated virgin females. Experiments with *D. melanogaster* by Muller and Settles (1927) and Demerec and Kaufmann (1941) have indicated that doses of X rays approaching the limit of tolerance of the adult fly (ca. 5000–10,000 r) do not usually inactivate the spermatozoa, which fertilize the eggs and participate in zygote formation. Some of the fertilized eggs fail to hatch, death of the embryos being attributable in many cases to loss or duplication of sections of chromosomes in early cleavage mitoses (Sonnenblick, 1940). The abortive embryos constitute a class of so-called "dominant lethals," whose frequency can be determined by counting the number of eggs laid and the number from which larvae do not emerge.

Larvae hatching from eggs fertilized by irradiated spermatozoa may or may not carry detectable chromosomal rearrangements. The relative frequencies of the two classes resulting from any given treatment can be determined by either cytological or genetical techniques. The cytologic

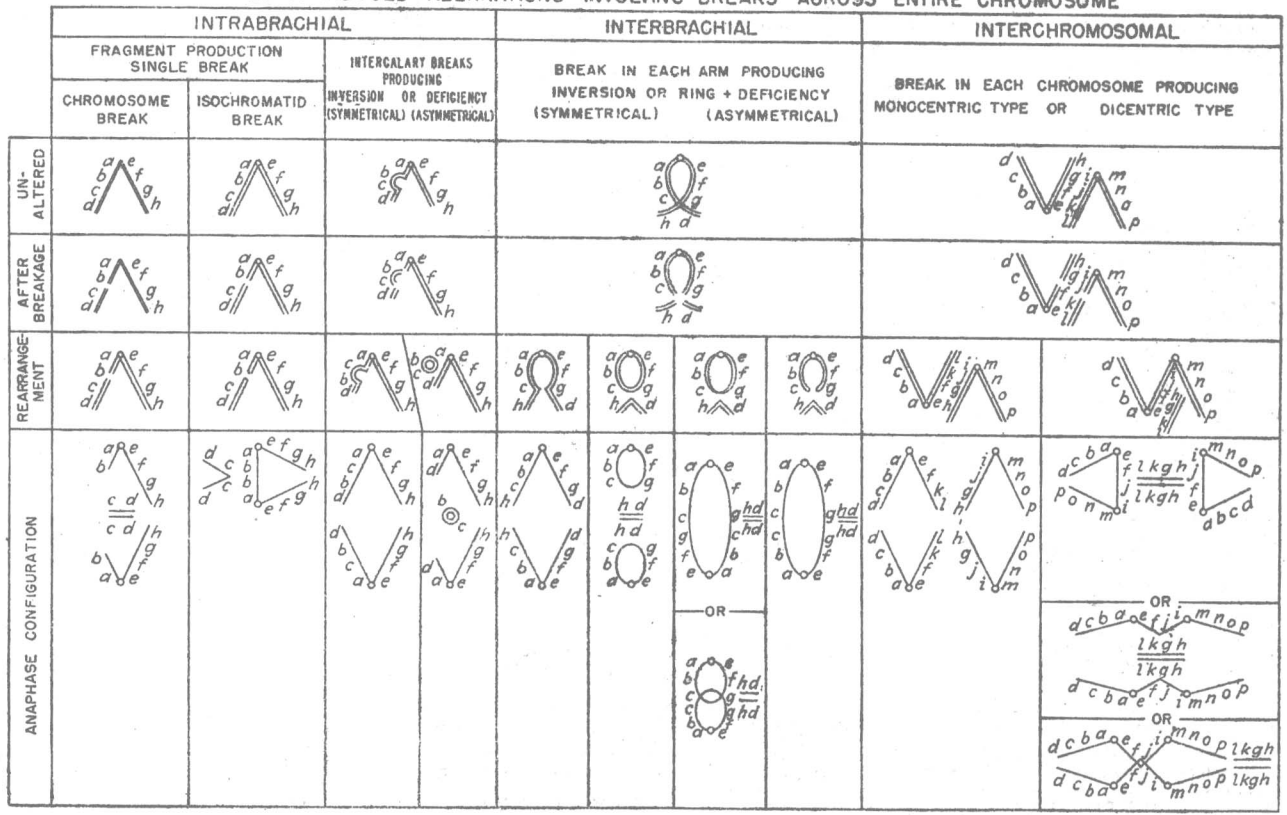

FIG. 9-3. Diagram of types of chromosome aberrations detected after irradiation of animal cells. Continued in Fig. 9-4; further description in text.

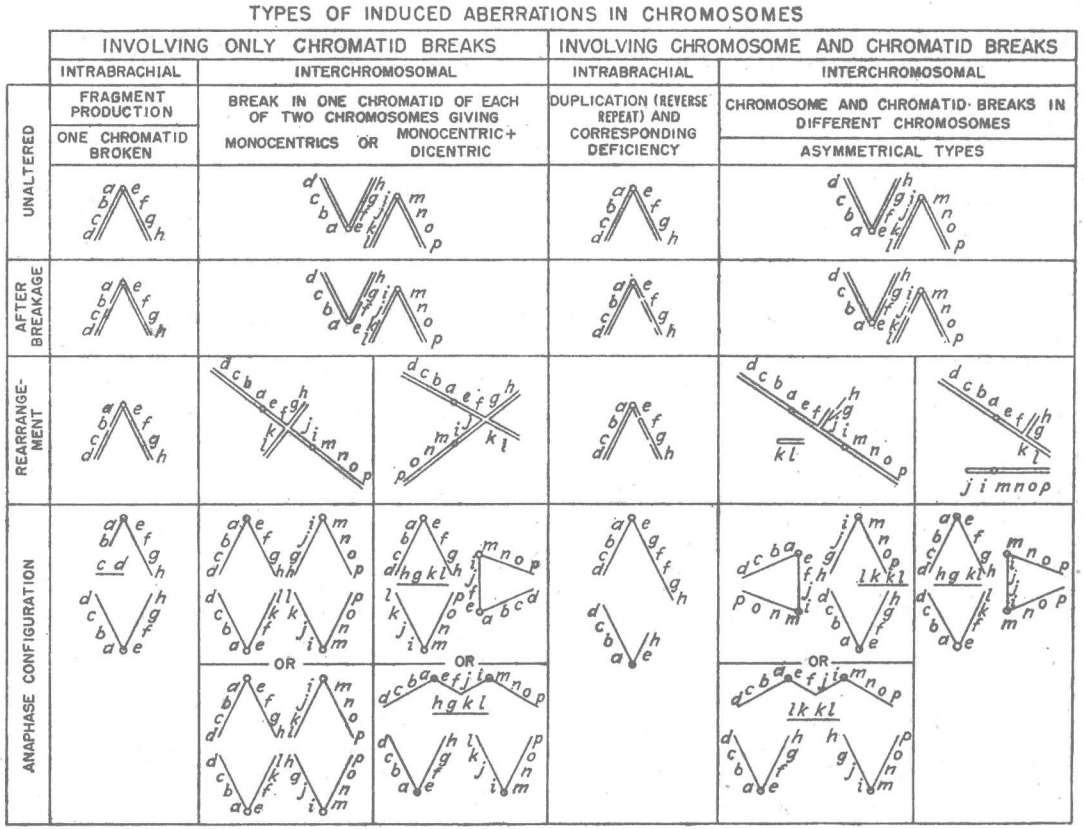

FIG. 9-4. Diagram of types of chromosome aberrations detected after irradiation of animal cells. Other types shown in Fig. 9-3; further description in text.

approach involves inspection of the salivary-gland chromosomes of the first generation (F_1) larval progeny of the irradiated fathers. Since analysis of a given rearrangement by this method is restricted to the chromosomes of a single individual, aberrations of special interest cannot be perpetuated for genetic analysis and subsequent experimental use. On the other hand, the large size and precise pattern of banding of the salivary-gland chromosomes (as shown in Figs. 9-5, 9-6) offer unparalleled opportunities for determining the complexity of a rearrangement and the positions of the breaks involved in its production—as was first demonstrated by Painter in 1933. Methods of preparing for cytological examination the aceto-carmine or aceto-orcein smears from which these photographs were made are briefly outlined in the "Drosophila Guide," by Demerec and Kaufmann (1950).

One limitation of the salivary-gland-chromosome method of diagnosis is the difficulty of detecting rearrangements that are restricted to proximal heterochromatic regions; these parts of the chromosome have poorly defined bands, and aggregate to form a so-called "chromocenter" (illustrated in Fig. 9-5a). Exchanges involving breaks in proximal heterochromatic regions can sometimes be detected in neuroblast cells of the larva by the pattern of somatic pairing of the chromosomes. Thus the cross-shaped configuration shown in the small inset of Fig. 9-5a (cf. Fig. 9-7b), results from the side-by-side association of unaltered second and third chromosomes, maternal in origin, with second and third chromosomes of the paternal set that had exchanged parts (reciprocal translocation) as a result of irradiation of the spermatozoon. Identification of intrachromosomal exchanges in neuroblast cells is difficult, but is sometimes possible because of the presence of constrictions, including those associated with the formation of the nucleoli, that are visible in late prophase stages (Fig. 9-7a, c, d, e). From these considerations it is apparent that a comprehensive quantitative study of induced rearrangements should include analysis of both salivary-gland and neuroblast chromosomes from the same larva. The labor involved in cytological examination of the neuroblasts is so considerable, however, that they are rarely utilized for this purpose. Before the advantages of salivary-gland chromosomes were recognized, cytological studies of genetically detected rearrangements were made exclusively on chromosomes of neuroblast cells of larvae or gonial cells of adults (see, for example, Dobzhansky, 1936; Stern, 1931).

Genetic techniques for determination of induced chromosomal exchanges were described by Muller and Altenburg (1930) and Dobzhansky (1929, 1930). Both intra- and interchromosomal rearrangements had previously been known in *Drosophila*. Sturtevant (1926) had shown that a reduction of crossing over in the third chromosome of *D. melanogaster* was due to inversion (rotation through 180° as a consequence of breakage

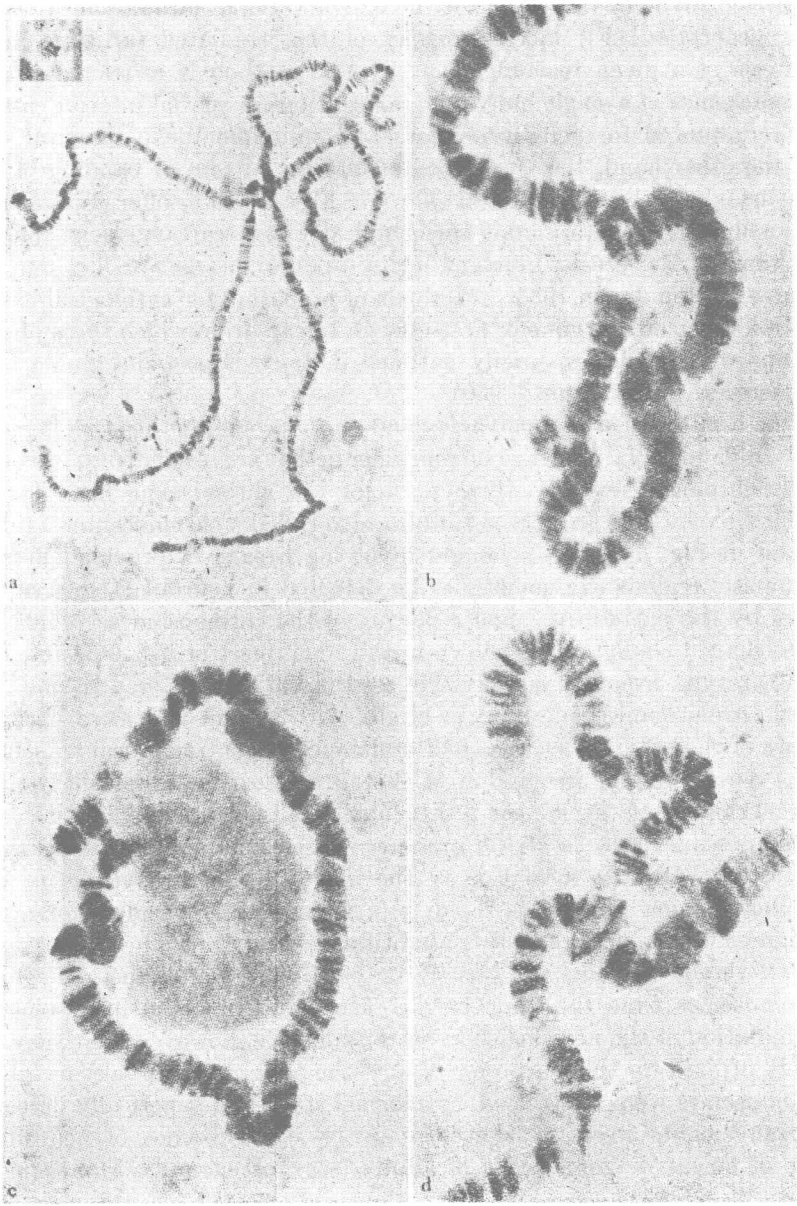

FIG. 9-5. Salivary-gland chromosomes of *Drosophila melanogaster*. Rearrangements detected in larval progeny of irradiated fathers. (*a*) Unaltered complement; chromosomes aggregated at their proximal heterochromatic regions to form the chromocenter. (Insert shows chromosomes of neuroblast cell, not from same individual but photographed to same scale as salivary-gland chromosomes.) (*b*) Transposition of section of right limb of third chromosome. (*c*) Inversion in X chromosome; inversion loop surrounds nucleolus. (*d*) Intercalary duplication (reverse repeat) in left limb of third chromosome. (*Photographs of preparations by the author.*)

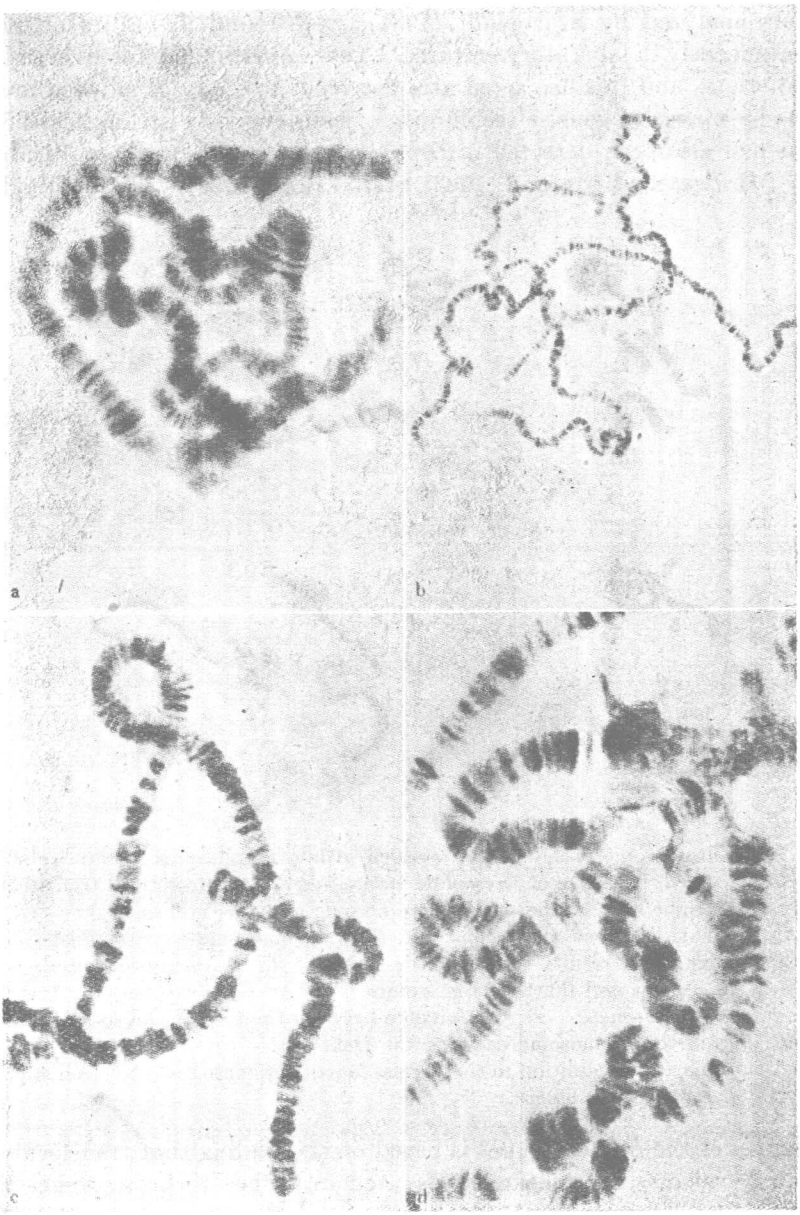

FIG. 9-6. Salivary-gland chromosomes of *Drosophila melanogaster*. Rearrangements detected in larval progeny of irradiated fathers. (*a*) Duplication of terminal section of left limb of second chromosome. (*b*) Reciprocal translocation between the Y chromosome and the right limb of the third chromosome. (*c*) Reciprocal translocation between the left limb of the second chromosome and the right limb of the third chromosome. (*d*) An inversion-translocation complex involving breaks in 2L, 2R, and 3L. (*Photographs of preparations by the author.*)

and reinsertion) of a section of that chromosome. This inversion, and others analyzed by Sturtevant (1931), were found in nature or arose spontaneously in laboratory cultures. Determination of crossover values is laborious, and this has acted as a deterrent to study of induced inversions by means of genetic techniques. Spontaneously arising translocations had also been detected in *Drosophila* by genetic analysis (Bridges, 1923; Bridges and Morgan, 1923; Stern, 1926, 1929). The method of

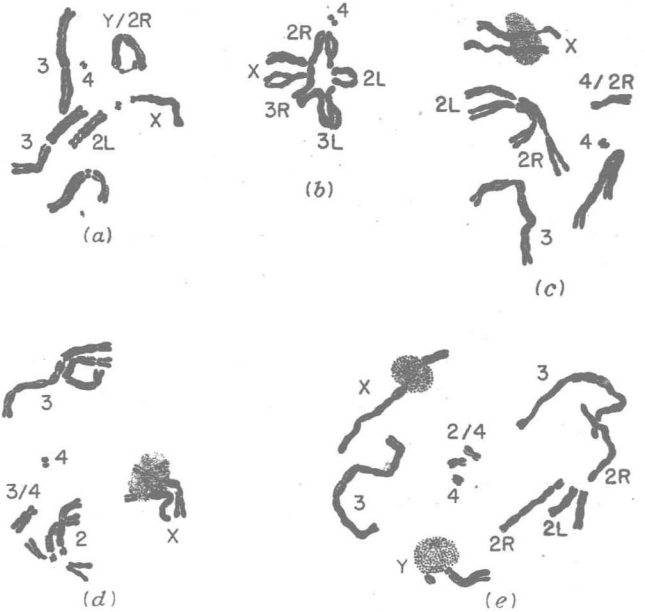

FIG. 9-7. Chromosome exchanges as revealed in late prophase or metaphase stages in neuroblast cells of larvae of *Drosophila melanogaster*. (*a*) Reciprocal translocation between the right limb of the second chromosome and the Y chromosome (2R/Y). (*b*) An exchange between the second and third chromosomes; characteristic cross-shaped configuration results from somatic pairing. (*c*) A reciprocal translocation between the second and fourth chromosomes. (*d*) An exchange between the third and fourth chromosomes. (*e*) An exchange between the second and fourth chromosomes; the fourth chromosome carrying the translocated tip of the second is represented in duplicate, in addition to the normal fourth chromosome. Nucleoli stippled. (*Original drawings by the author.*)

diagnosis of the induced types is based on the finding that translocations produce linkages between genes located in different chromosomes that would normally segregate independently.

In the genetic, as in the cytologic, method for detection of translocations, males are usually irradiated and mated with untreated virgin females. If the males are wild type, they may be crossed with females whose chromosomes carry marking mutant genes. In a typical experiment of this type, outlined in Fig. 9-8, irradiated males having wild-type

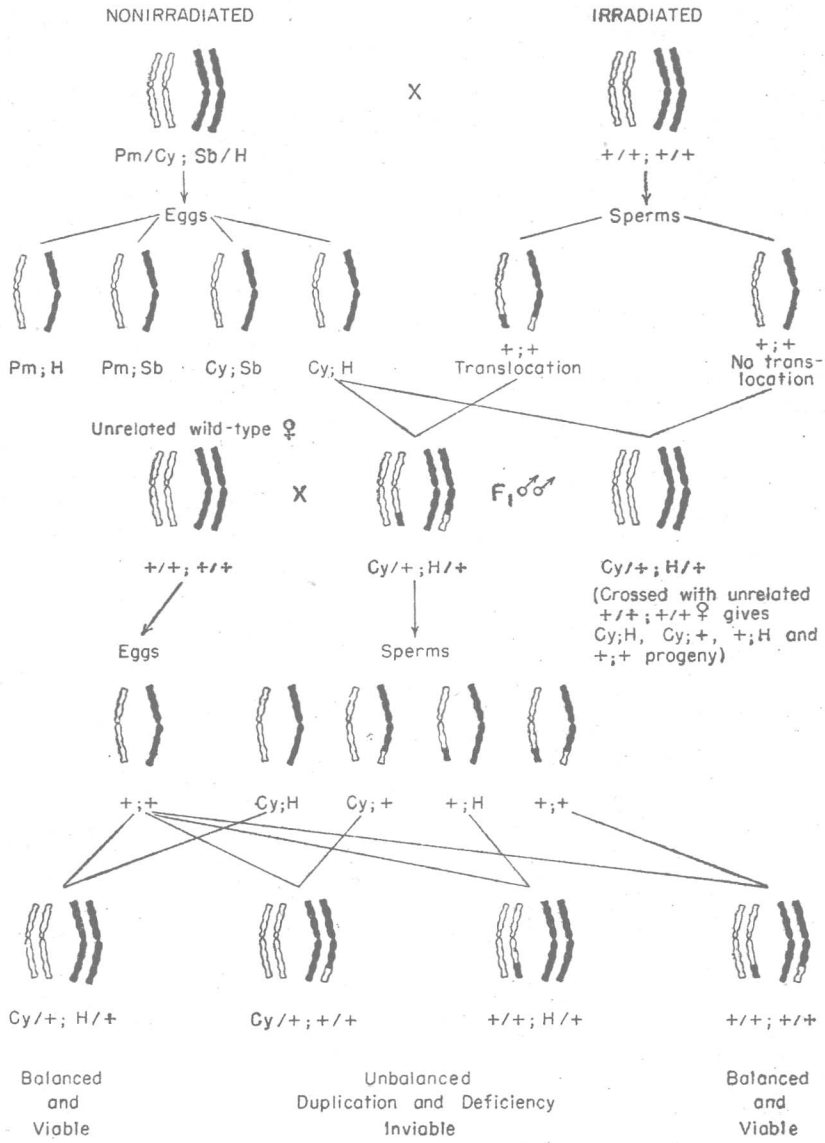

FIG. 9-8. Diagram of the genetic method for detecting reciprocal translocations between the second and third chromosomes in *Drosophila melanogaster*. Second chromosomes shown at left in outline, third chromosomes at right in solid color. The chromosomes with dominant marking genes carry inverted sections which are not indicated in the diagram. Only crosses involving Curly and Hairless (*Cy;H*) are detailed, but similar results are obtainable with Plum and Hairless (*Pm;H*), Plum and Stubble (*Pm;S,b*) and Curly and Stubble (*Cy;Sb*).

second and third chromosomes (represented by the symbols +;+) are mated with females whose second chromosomes carry the dominant markers Curly (*Cy*) and Plum (*Pm*), and the third chromosomes the dominants Hairless (*H*) and Stubble (*Sb*). The heterozygous F_1 flies, which are of four types with respect to the dominant marking genes—namely, *Cy;H*, *Cy;Sb*, *Pm;H*, and *Pm;Sb*—are mated individually with unrelated wild-type flies of the opposite sex. Figure 9-8 represents a cross between heterozygous F_1 males and wild-type females. The presence or absence of translocations between the second and third chromosomes is determined by examination of the F_2 cultures. If no translocation has been induced by irradiation, each of the second and third chromosomes of the F_1 males, whether paternal or maternal in origin, will carry a normal complement of genes. Independent assortment at meiosis will yield four types of spermatozoa, with respect to the mutants under consideration, which will produce, by fertilization of eggs bearing the wild-type chromosomes, four kinds of F_2 progeny in approximately equal numbers (for example, from the cross *Cy;H* ♂ by +;+ ♀ *Cy;H*, *Cy;+*, *+;H*, and +;+ males and females). If, on the other hand, an induced translocation is present, only the maternally derived second and third chromosomes will carry an unaltered complement of genes, since those of paternal origin will have exchanged parts with each other. Independent assortment at meiosis will yield four types of spermatozoa, but two of them will carry some genes in duplicate and be deficient for others (bottom row of Fig. 9-8). Eggs fertilized by such spermatozoa will not as a rule give rise to viable progeny, although a duplication or deficiency zygote may occasionally survive to produce an individual possessing special somatic characteristics. Eggs fertilized by the other two types of spermatozoa (those carrying the second and third chromosomes with the dominant markers, and those carrying the two chromosomes that have exchanged parts), both of which transmit a complete set of genes, will produce viable progeny. The occurrence, in the cross illustrated, of only two classes of F_2 progeny—namely, *Cy;H* and +;+—will thus serve as an index to the induction of a reciprocal translocation.

Translocations may be detected in a similar manner by irradiating males whose chromosomes carry dominant marking genes, and mating them with nonirradiated wild-type females. This procedure is described in detail by Dobzhansky (1936).

Another technique for the detection of translocations involving a particular chromosome is based on phenotypic modification accompanying change in position of a specific gene. Thus the cubitus interruptus (*ci*) position effect in *D. melanogaster*, which alters the normal pattern of wing venation, is caused by a translocation involving the fourth chromosome, whereby the dominance of the wild-type allele of cubitus interruptus is weakened (Dubinin and Sidorow, 1934). Using this criterion of assay,

Eberhardt (1939) irradiated flies carrying a normal fourth chromosome and determined the frequency of translocations from the proportion of progeny showing interruptions in the cubital vein.

In a more elaborate experiment designed to disclose exchanges among all four chromosomes of an irradiated spermatozoon, Patterson, Stone, Bedichek, and Suche (1934) mated irradiated wild-type males with females having attached-X chromosomes homozygous for the mutant gene yellow ($\widehat{yy}$), the second chromosome homozygous for brown (*bw*), the third for ebony (*e*), and the fourth for eyeless (*ey*). The heterozygous F_1 males were backcrossed individually to $\widehat{yy}$; *bw*; *e*; *ey* females.

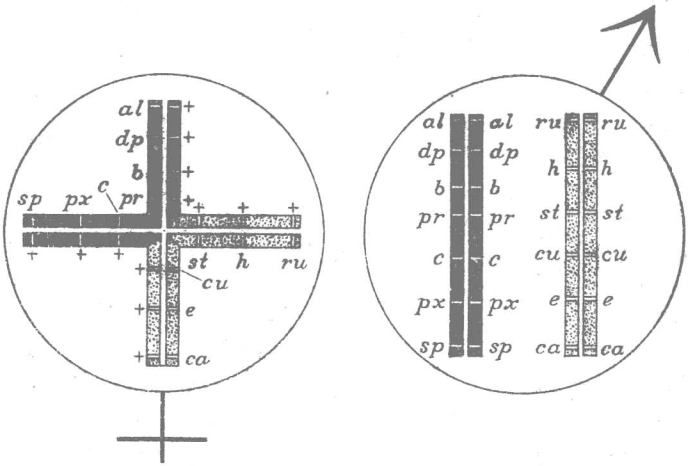

FIG. 9-9. Diagram illustrating the genetic technique for determining points of exchange between chromosomes in *Drosophila melanogaster*. A translocation-carrying female heterozygous for a series of genes (left) is crossed to a male free from the translocation and homozygous for the same genes (right). (*From Dobzhansky, in Duggar's "Biological Effects of Radiation,"* 1936.)

Since these males carried X chromosomes received from their irradiated fathers, many different types of reassociation could be detected by examination of F_2 cultures, e.g., X;2, X;3, X;4, 2;3, 2;4, 3;4, X;2;3, X;2;4, X;3;4, 2;3;4, and X;2;3;4. Some duplication and deficiency types were also viable and could be detected by criteria that will be indicated presently. Translocations involving the Y chromosome were not detected in these experiments because the females that came from eggs fertilized by Y-bearing spermatozoa were not tested.

When translocations have been diagnosed by such genetic methods, they can usually be perpetuated in cultures, and the positions of the breaks involved in the rearrangement can subsequently be determined by either of two procedures. The most informative and least laborious is examination of salivary glands of individuals carrying the translocation in heterozygous condition. The alternative method, which was the first